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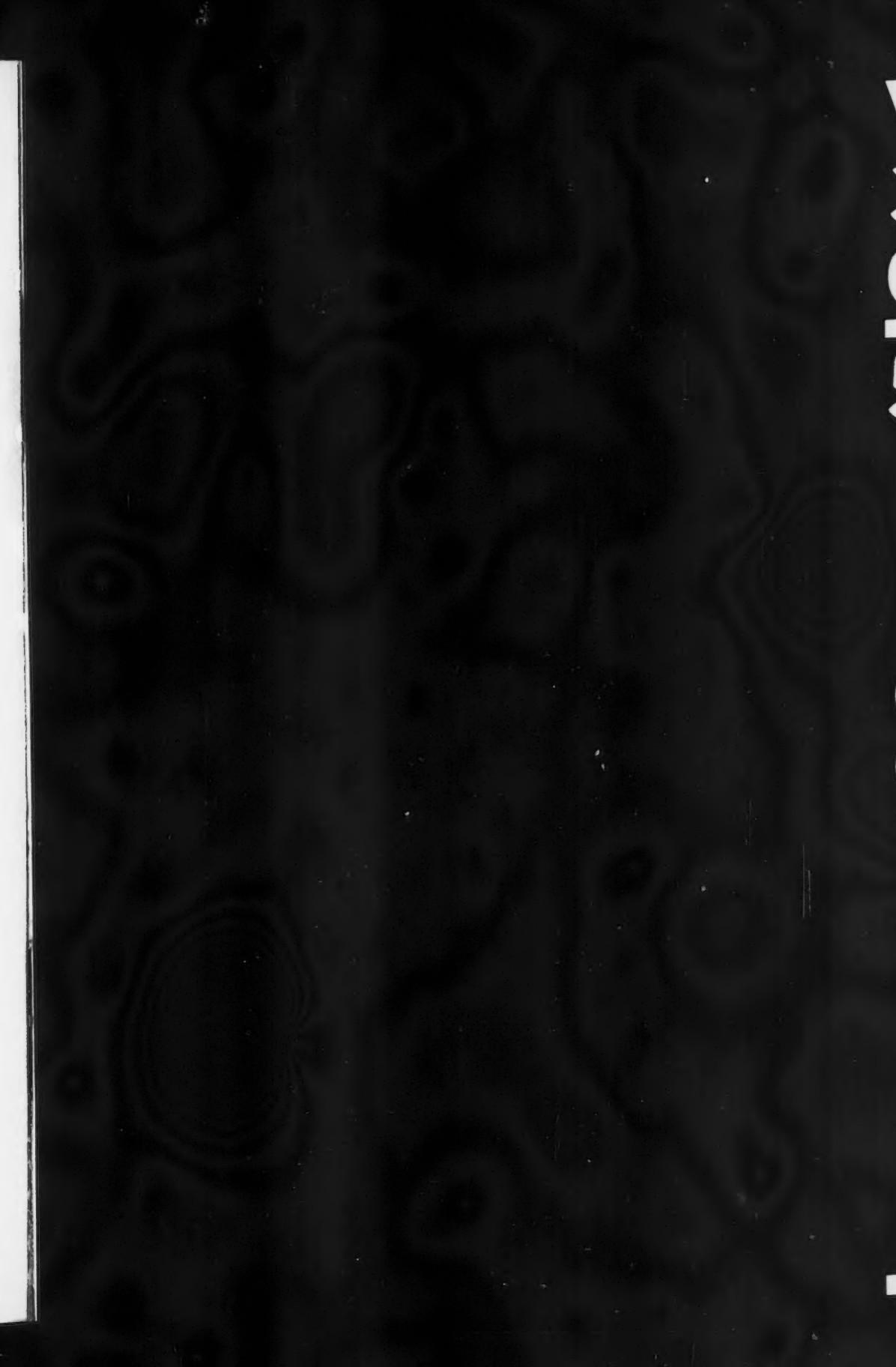
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THE RATE OF DIGESTION OF BLOOD IN MOSQUITOES. PRECIPITIN TEST STUDIES¹

BY A. S. WEST² AND G. S. ELIGH³

Abstract

This paper describes the use of the serological procedure known as the precipitin test to study the rate of digestion of host blood in mosquitoes. In the laboratory, at constant temperatures, precipitin reactions were obtained on blood meals of *Aedes aegypti* eight days after engorgement when the mosquitoes were held at 11° C. but only one or two days after engorgement when the holding temperature was 27° C. Field studies with *A. hexodontus* in northern Manitoba also showed that temperature has a strong influence on the rate of blood digestion. Pertinent literature is reviewed and the limitations of the precipitin test are discussed. Until now little attention has been devoted in Canada to possible disease transmission by mosquitoes. Increasing interest in mosquitoes as vectors of the encephalitides and proved transmission of western equine encephalitis by *Culex restuans* in Manitoba suggest the importance of further knowledge of mosquito physiology.

Introduction

The apparent necessity of blood for adult females of many mosquito species has naturally led to extensive studies on blood meals, particularly their digestion and relationship to egg development and oviposition. Much of the pertinent literature has been reviewed recently by Bates (1). The rate of blood digestion is an important factor not only in the physiology of mosquitoes but also in studies of host relationships. The speed of digestion is related to the frequency of biting, which in turn has a bearing on the nuisance and irritation created by mosquitoes. More particularly, the speed of digestion may be a factor in the efficiency of disease transmission.

Fisk (6) has recently pointed out that since the etiological agents of diseases transmitted by mosquitoes are ingested by the insects before transmission can occur, "Extensive physiological investigations of the culicid digestive system may be expected to provide fundamental bearing on the relationships which mosquitoes have with these several disease producing agents".

The rate of blood digestion is of practical importance in relation to its limiting of the effectiveness of the precipitin test in identifying mosquito blood

¹ Manuscript received December 7, 1951, and, as revised, July 4, 1952.

The results reported herein were obtained from studies sponsored by the Defence Research Board; the Division of Entomology, Science Service, Canada Department of Agriculture; the Research Council of Ontario; and Queen's University. These studies are part of a program of investigations on biting flies carried out by the Division of Entomology and cooperating agencies on behalf of the Defence Research Board.

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meals. The writers have been using the precipitin test in studies on the blood feeding habits of Canadian arctic and subarctic biting flies. The period of time after engorgement during which positive precipitin reactions can be obtained is one factor conditioning the success of this project. The longer this period the more opportunity there is for collecting suitable female mosquitoes.

This paper reports the results secured when the precipitin test was used to determine the effect of temperature on the rate of digestion of blood. At Kingston, Ont., *Aedes aegypti* (L.) was used for laboratory studies; at Churchill, Man., field studies were conducted with *Aedes hexodontus* Dyar.

Methods and Results

In the laboratory adults of *A. aegypti* were allowed to emerge in small cages, approximately 10 in. in each dimension. After a four- to five-day period during which water and 5% glucose was supplied, a human blood meal was offered. An arm was held against the cage and engorgement without disturbance was permitted. Females with fully distended abdomens were removed from the cage and placed in a glass battery jar that was closed with a cheese-cloth cover. Water and 5% glucose were provided. The jars were placed in darkness at various constant temperatures, including $26 \pm 1^\circ$, $15 \pm 1^\circ$, $11 \pm 1^\circ$, and $6 \pm 1^\circ$ C.

Smears were prepared from samples of mosquitoes removed from jars at intervals of 24 hr. after engorgement. The manner of preparation of smears and the procedure of precipitin testing are described in a paper by Eligh (5). Positive and negative controls were run with tests on each group of smears. No difficulty with false positives was encountered.

During the summer of 1950, several series of smears were prepared at Churchill, Man. Specific identification of all individuals fed and smeared was not possible. Samples of the natural population used were examined by Mr. W. E. Beckel at Churchill and showed a preponderance of *A. hexodontus* with only an occasional individual of another species.

In the field adults were allowed to bite on an exposed forearm. Fully engorged individuals about to fly were captured in a vial and transferred to a cloth-covered cage with a xylonite front. The cages were approximately 12 in. by 12 in. by 18 in. A Petri dish containing moss and water was placed in each cage. Two cages were also provided with raisins and 5% glucose. There was no evidence that the presence of raisins or sugar in any way affected the rate of blood digestion. The cages were placed on the ground at the base of a spruce tree and were shaded from the direct sun by overhanging branches. Maximum and minimum air temperatures were recorded in a standard instrument shelter about one-quarter mile from the cages.

Females were removed from the cages and smears prepared at intervals of 24 hr. after engorgement until all living mosquitoes had been used. All precipitin tests were done at Kingston, Ont., during the winter months. Storage of smears has no effect on the precipitin reaction.

Aedes aegypti in the Laboratory

The results of a series of tests with *A. aegypti* held at various constant temperatures after feeding are given in Table I. The effect of temperature on the rate of digestion of blood is clearly evident. The temperature at

TABLE I

THE EFFECT OF TEMPERATURE ON THE RATE OF DIGESTION OF HUMAN BLOOD BY
Aedes aegypti (L.) AS INDICATED BY PRECIPITIN TESTS

Temperature, ° C.	Age of blood meal in days	Number of smears tested	Positive precipitin tests, %
27 ± 1	1	10	100
	2	60	8.3
	3	45	0
15 ± 1	2	10	100
	3	10	0
	4	10	0
11 ± 1	3	10	100
	4	10	100
	5	10	100
	6	10	100
	7	10	100
	8	10	100
	9	All dead	—
	4	10	100
	5	All dead	—

which mosquitoes are reared in the Kingston laboratory is approximately 26° C. At 15° C. the mosquitoes were active and flying when viewed by artificial light. Apparently water and sugar were utilized. At 11° C. and 6° C. the mosquitoes were not active. After engorgement the limit of life at 11° C. was eight days. As already stated, all cages were held in darkness.

Aedes hexodontus in the Field

Four lots of *A. hexodontus* females were fed on humans and placed in cages as described. The cages were set up during a period of 17 days. Two cages provided sufficient mosquitoes for series of smears extending for four and five days. All smears prepared from blood meals digested from one to five days gave positive precipitin reactions with human antisera. Temperatures prevailing during the exposure of these two cages were relatively low, with maxima of about 70° F.

By fortunate circumstance two other lots of engorged mosquitoes were exposed to differing temperatures and each cage furnished a more extended series of smears. The results of precipitin tests on smears of these series are given in Table II. The maximum and minimum temperatures show that Lot 2 was exposed during a period of relatively cool weather. A three-day warm spell occurred early during the exposure period of Lot 4. These higher

TABLE II

THE EFFECT OF TEMPERATURE ON THE RATE OF DIGESTION OF HUMAN BLOOD BY *A. hexodonius* DYAR

Lot 2				Lot 4					
Temperature ¹ , ° F.		Age of blood meal in days	Number of smears tested	Positive precipitin tests, %	Temperature, ° F.		Age of blood meal in days	Number of smears tested	Positive precipitin tests, %
Max.	Min.				Max.	Min.			
55.0	34.0	0	10	100	58.5	38.8	0	0	—
57.0	35.0	1	10	100	62.5	49.0	1	0	—
60.0	42.0	2	0	—	80.2	50.8	2	0	—
61.0	41.0	3	0	—	83.6	63.1	3	0	—
65.3	44.0	4	10	100	84.0	41.0	4	20	65
56.5	40.0	5	10	90	65.4	40.0	5	20	35
58.5	37.0	6	10	100	61.0	39.0	6	20	15
58.0	31.0	7	16	88	64.5	42.5	7	20	10
60.0	34.0	8	10	50	63.5	43.0	8	20	0
67.0	50.0	9	10	0					
71.0	43.0	10	10	0 ²					
58.5	38.8	11	10	0 ³					

¹ Maximum and minimum temperatures were read at 0900 hr. for the preceding 24 hr. period.² Positive Meyer test for blood, three out of four smears.³ Positive Meyer test for blood, two out of three smears.

temperatures evidently speeded blood digestion significantly. Only 10% positive precipitin tests were secured after seven days, during part of which time high temperatures were recorded. In contrast, when lower temperatures prevailed throughout the exposure period, 88% positive tests were secured after seven days and 50% after eight days.

A duplicate of Lot 4 was run at the same time. No smears were prepared but an opportunity to feed was offered daily. The first engorgement occurred on the sixth day.

In the Canadian north, engorged mosquitoes are extremely difficult to find in nature. It is apparent that during cool weather there would be a longer period during which an engorged individual might be collected.

Discussion

Since the studies of Uhlenhuth and co-workers in 1908 (13) a number of investigators have made use of the precipitin test to identify the source of blood meals of biting flies. Eligh (4) has reviewed the pertinent literature recently. Some of these studies have contributed, indirectly, information on the rate of blood digestion. Several workers have referred to the digestion time factor as related to the performance of precipitin tests (3, 9, 11).

Several methods other than serological have been used for studies of rate of digestion of blood by mosquitoes. Observational studies by deBuck and co-workers (2), working with *Anopheles maculipennis* Meig., showed racial differences in rate of digestion of blood at given temperatures. Shlenova's (12)

detailed studies on *Anopheles messeae* Falleroni were observational. Shlenova found that digestion was accelerated by higher temperatures up to 30° C. but was retarded at 35° C. High humidity accelerated digestion at temperatures above 15° C. He also reported that digestion occurred more rapidly in small mosquitoes than in large ones.

Huff (8) used histological methods to study rate of digestion in *Culex pipiens* L. Serial sections made at various intervals after feeding showed that digestion began at the stomach wall and proceeded inwardly. Huff's studies were not related to temperature.

Fisk (6) and Fisk and Shambaugh (7) have reported recently on studies of proteolytic digestion in *Aedes aegypti*. Fish and Shambaugh (7) conclude "—it is likely that complete digestion of the blood by a living mosquito requires nearer 48 than 72 hours." Fish has indicated in correspondence that temperatures for the latter studies ranged between 70° and 80° F.

Each technique which has been used for the study of digestion of blood by mosquitoes and other blood-sucking arthropods has its advantages and disadvantages. The precipitin test has been most widely used, although not extensively for this one purpose.

Certain limitations are inherent in the use of the precipitin test. Eligh (5) has shown that antisera of high titer and power to precipitate are necessary. Such antisera are not always easily produced. In the present studies the precipitin test depends upon the reaction between blood antigens and specific antisera prepared to these antigens. After digestion has proceeded to a certain degree the antigens are so altered that they no longer react with their homologous antibodies to form a precipitin ring. This development occurs at a time when partially digested blood is still present.

The precipitin test will not then indicate when digestion of a blood meal has been completed. That blood fractions are still present when precipitin reactions can be obtained no longer is shown by use of the extremely sensitive Meyer reduced phenolphthalein test¹ for blood. A positive Meyer test was secured on three out of four *A. hexodontus* smears prepared 10 days after engorgement and on two out of three smears prepared on the 11th day. Precipitin reactions for smears of the same series were secured for only 50% of smears prepared on the eighth day after engorgement (Table II). It is probable that the protease activity measurement as used by Fisk (6) might give a more accurate indication of completion of blood digestion than would any other known method.

Factors other than temperature may affect the rate of digestion of blood. The possible influence of light intensity on the degree of activity and hence on the rate of blood digestion is recognized. Shlenova's reference to the accelerating effect of high humidity has been mentioned. In the present laboratory studies humidities were uniformly high. In the field, humidities were highest during the period of lower temperatures. Species differences and differences in host bloods undoubtedly exist. These differences are being studied.

Until recently the field of medical entomology has not been regarded in Canada as of major importance. Studies have for the most part been limited to a few specific problems. At the present time there is an awakening interest in possible disease transmission by insects. Biting flies are a natural subject of study since they occur in extremely large numbers in many parts of Canada. World-wide interest in mosquitoes as vectors of the encephalitides and proved transmission of western equine encephalitis by *Culex restuans* Theo. in Manitoba (10) suggest the importance of further knowledge of mosquito physiology.

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EFFECTS OF SOME HORMONES ON THE BEHAVIOR OF SALMON FRY¹

BY WILLIAM S. HOAR², DIXON MACKINNON³, AND ALINE REDLICH⁴

Abstract

Chum and coho salmon fry, when immersed in solutions of methyl testosterone, synthetic thyroxine sodium, or the antithyroid drug, thiourea, do not show any new or different patterns of behavior. Chum salmon fry do, however, show quantitative changes in the rate of swimming and in the intensity of schooling. Less marked variations are seen in aggregating behavior of the treated coho. Control chum fry swim in the brightest part of a horizontal light gradient while coho fry, coho smolt, and trout prefer darker areas. Minor changes in this photoresponse, observed in fish treated with drugs, are probably produced by variations in the amount of swimming.

Introduction

When all the facts have been assembled, each of the many movements and activities of a fish will be seen as a part of some particular innate behavior pattern. These numerous and varied activities are evidently organized in a hierarchical manner with respect to any particular instinctive action (12, 16). Tinbergen (16) has discussed this hierarchical system in the behavior of the stickleback and shown how the change in gonadic hormone level in the blood will activate nervous centers and initiate generalized appetitive behavior (migration). This, in turn, will be followed by progressively more specialized acts as the appropriate releasers are encountered and the innate releasing mechanisms activated until the final consummatory reproductive act is performed. External environmental variables guide and direct the different activities involved.

An understanding of the behavior of Pacific salmon is not only academically interesting but also of fundamental importance for fisheries exploitation and management. Schooling, migration, and the responses to various gradients in the environment must be understood if predictions with respect to the fisheries are to be made. Before predictions of the behavior of any species can be attempted it will be necessary to (a) catalogue, in minute detail, behavior patterns with their associated releasers, (b) understand the effect of physiological conditions (hormone secretion, hunger, water balance) on the activation of nervous centers, and (c) understand the directing and controlling factors of the external environment.

Pacific salmon, migrating to the sea as juveniles, frequently have very active thyroid glands (8). They return to fresh water as adults when the gonads are showing signs of maturation. Thyroid and gonad hormones may

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be involved either directly or indirectly in activating nervous centers which lead to the migratory behavior. In this investigation, an attempt is made to determine whether thyroxine and testosterone change or modify some of the behavior patterns of chum (*Oncorhynchus keta*) and coho (*O. kisutch*) salmon fry. The rate of swimming, schooling, and aggregating behavior and phototactic responses were chosen for study. There is adequate evidence in the literature that, in several species of fish the schooling and aggregating behavior does change as the animals become sexually mature (13). Likewise gonad hormones have been shown to increase activity (15) and rate of metabolism in fish. The photoresponse was selected for analysis because of the striking difference in this response shown by stream-dwelling coho and migrating juvenile chum salmon.

Materials and Methods

Experiments on schooling and aggregating of salmon fry were carried out during the spring and summer of 1950 at the Nile creek field station of the Pacific Biological Station, Fisheries Research Board of Canada. Chum salmon fry (25 mm. to 45 mm.) were obtained from Nile creek and maintained in the hatchery until required. Coho fry (40 mm. to 65 mm.) were captured in Nile creek, Brunell creek, or Cook creek and maintained in holding boxes floating in Nile creek. All fish were fed three times daily on a mixture of minced canned salmon and pablum.

Experiments on photoresponse and rate of swimming were done in the University laboratory during the spring and summer of 1951. The fry used were of comparable size to the 1950 stock and were captured from a number of different streams of the Nanaimo area of Vancouver island and from streams of the lower mainland. Coho smolt (100 mm. to 120 mm.) and trout (*Salmo gairdneri*) of comparable size were obtained from some of the same locations, and also compared for normal responses to light. The stocks of fish were maintained in the University hatchery and fed on the minced salmon and pablum.

Fish were treated for periods of from one to two weeks by immersion in hormone solutions. Ten liters of constantly aerated solution were used for each group of 30-35 fry. Fish were fed sparingly but regularly and the solutions changed three times per week. The aquaria were maintained at the same temperature by placing them on a concrete basement floor (Nile creek) or in troughs of running water (University). Variations in temperature are noted in the section on results.

Different groups of fish were treated as follows:

- (a) 1 : 2,000,000 synthetic thyroxine sodium B.D.H.
- (b) 1 : 1000 thiourea (Amend Drug)
- (c) 1 : 3,200,000 methyl testosterone (Oreton-M, Schering Corp.)
- (d) River water control maintained under identical conditions to the above.

Procedure for Schooling and Aggregation Studies

Schooling or aggregating behavior was studied by observing fish in two adjacent circular galvanized iron wash tubs, each of which was divided into four equal sections by painting black lines on the interior, bottom, and sides. The tubs measured 70 cm. in diameter and had a centrally located vertical overflow pipe which maintained the depth at 17.5 cm. An electric lamp was fixed directly above the center of each tub. This was the only light source in the laboratory and it provided a relatively equal intensity of about 75 ft-c. at the water surface. Water entered the tubs through a vertical pipe, 1 cm. in diameter, attached to the wall (6).

Twelve fish were placed in a tub on the evening preceding the experiments. The water was turned on and the room darkened. The following morning the water was turned off and the lamps were turned on. One hour later observations were commenced from a vantage point, out of sight of the fish.

The observation procedure was to note the number of fish in each quadrant of the tub at one-minute intervals. A stop watch was used to make the time intervals exact. After the first eight one-minute observations the fish were disturbed by passing a book between the light source and the fish. Recording was then continued for another seven minutes (15 minutes in all). Chi-square values for each one-minute observation totaled after eight observations and after 15 observations gave a measure of the departure from uniform distribution and consequently a measure of the grouping as well as the effect of disturbance on this grouping. A high chi-square indicates a greater amount of grouping than a low chi-square.

After 15 min. of observation water was run through the vertical inlet pipe on the wall of the tub. This pipe was perforated by a series of holes along one side (6). Thus, it not only provided a means of changing the water but also created a counter clockwise current which afforded an opportunity to observe rheotactic responses. This experiment was repeated every hour. Between experiments the light was left on, the laboratory vacated, and the fish were free from any outside disturbances. Temperatures varied with the temperatures of the Nile creek water from about 9.0° C. in early June to 16.0° C. in late August.

By using two tubs and observing one on the half minute and the other on the minute it was possible to study two groups in the time necessary to study one group. It is felt that this provided a better means of detecting qualitative differences in behavior and also helped to offset quantitative discrepancies. Qualitative comparisons of the different groups were made on activity, vertical distribution, and response to current.

Procedure for Measuring Responses to a Gradient in Illumination

Responses were measured in glass aquaria, 180 cm. long, 18 cm. wide, and 25 cm. deep. Water (approximately 2000 ml. per min.) flowed in at one end. A 10-cm. high overflow pipe, at the opposite end, maintained a constant level of water in the aquarium. The inflow and outflow ends were screened off by

perforated metal plates placed 15 cm. from the end walls. Narrow lines, painted at intervals of 25 cm. on the glass walls divided the main part of the tanks into six areas for ease in recording positions of fish. Two aquaria were used.

The aquaria were placed in separate dark rooms and covered with "frosted" glass plates. Four electric lights (one 60-watt lamp at either end and two 40-watt lamps between) were suspended above each aquarium so that a uniform light intensity of 40 ft-c. was obtained at the surface of the water under the "frosted" glass plates. With only one light (either of the end lamps) the intensities of illumination at the surface in different areas were 28, 20, 6, 1, < 1, < 1 ft-c. In other words, the light intensity in the illuminated end ranged from 28 ft-c. under the light to 6 ft-c. while the intensity in the opposite half of the aquarium was 1 or less than 1 ft-c.

The routine procedure adhered to in this study was as follows: (a) at 4 to 5 P.M. on any day 12 fish were placed in each aquarium, the water flow adjusted to approximately 2000 ml. per min. and the lights turned off; (b) at 9 A.M. the following day one end light was turned on; (c) at 10 A.M. the number of fish in each area was recorded at 30 sec. intervals for 15 min. (360 positions); (d) at the end of the recording this light was turned off and the light at the opposite end turned on; (e) one hour later a similar record was made and then all four lights turned on; (f) one hour later positions were again recorded and then the water flow turned off; (g) one hour later a final record was made. Each group of fish was tested on two successive days the only variation in procedure being that the order of turning on the end lamps was reversed from day to day.

Procedure for the Comparison of Swimming Speeds

The schooling chum fry frequently swim to and fro in the long aquarium. This activity is often carried on with such regularity and for such long periods that swimming speeds can be precisely recorded with a stop watch (6).

Records of swimming speeds were made during the course of the above experiments. Under uniform illumination, following the recording described under steps "f" and "g" (above), time required for a member of a travelling school to pass two neighboring lines on the aquarium (distance of 25 cm.) was recorded with a stop watch. In general, 20 such readings (in rapid succession) were made on any one occasion.

Results

Schooling and Aggregation

The total number of experiments on each treatment group varied (chums—37 on thyroxine, 40 on thiourea, 31 on testosterone and 35 controls; coho—34 on thyroxine, 38 on thioruea, 20 on testosterone, and 27 controls). Consequently only 20 experiments from each group were used for the statistical analysis. These experiments were selected by means of random numbers. Although this treatment eliminated a large number of observations it was the simplest means of compensating for the varying number of experiments.

Actually the picture was not essentially changed when all data were used and the chi-square values reduced to the same relative levels by taking appropriate fractions of them. However, the data presented here are strictly comparable on the basis of observations made.

The results show that the chum migrants treated with thyroxine or testosterone show a less intense grouping than the thiourea-treated or untreated fish (Fig. 1). Thiourea, on the other hand, increases the intensity of grouping in chums. The chi-square analysis can only be used to measure departure from uniform distribution. Qualitative observations, however, show that chums form true schools (6). The results also show (Fig. 1) that disturbance increases the schooling tendency of chums under all treatments. Qualitative

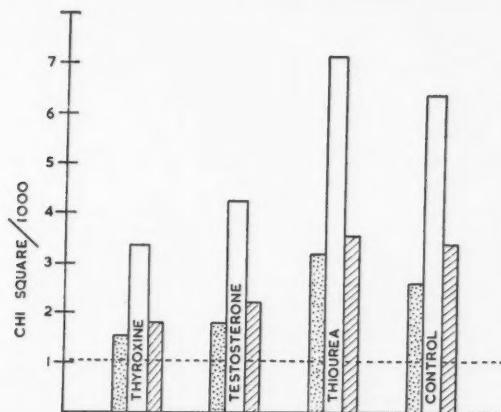


FIG. 1. Effect of hormone treatment on the schooling of chum fry. Plain bar, total response (15 min.); stippled bar, response before disturbance (7 min.); cross-hatched bar, response after disturbance (7 min.); broken horizontal line, 99% probability level for total response.

observations show that the thyroxine-treated and testosterone-treated chums display more activity and swim closer to the surface than thiourea-treated or control fish. Thiourea-treated and control chums almost invariably school near the bottom of the tub. Thiourea-treated chums also showed an alarm reaction to surface ripples, floating objects, and erratically moving fish.

Results of the coho experiments (Fig. 2) show that these fish group considerably, but the grouping intensity is not nearly as great as that of the chums (Fig. 1). Qualitative observations show that this grouping is not a true school but rather an aggregation (6). This aggregating tendency is greatest in the thiourea-treated cohos. Hormones produced little change in the coho response. Under all treatments the aggregating tendency of cohos is enhanced by disturbance (Fig. 2). No major differences in swimming, vertical distribution, or exaggerated responses to stimuli arising from moving objects or other fish were noticed in the coho experiments.

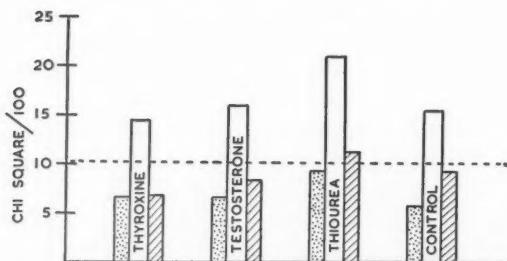


Fig. 2. Effect of hormone treatment on the grouping of coho fry (legend as in Fig. 1).

Responses to Gradients of Illumination

These data have been analyzed by comparing the mean number of fish counted in each half of the aquarium during the 30 min. of observation. These means are compared statistically in Tables I, II, and III and presented graphically in Fig. 3. All fish were treated from 7-14 days. It will be noted that a temperature range is given for the various groups of experiments. The

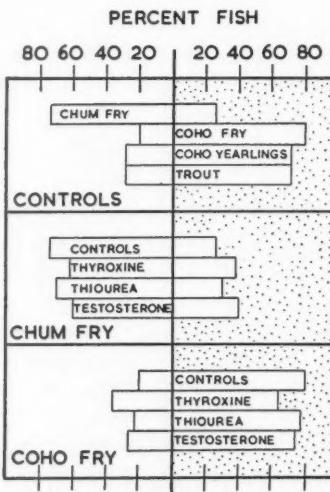


FIG. 3. Percentage of fish in the illuminated or dark (stippled) half of the aquarium based on total response of all fish studied.

fish were subjected to the normal variations in the temperature of the running water in the hatchery. This rose gradually during the summer. The results are comparable in that groups of thyroxine-treated and control fish were observed on the same days while thiourea-treated and testosterone-treated groups were observed together on the alternate days.

TABLE I

COMPARISON OF RESPONSES OF THREE SPECIES OF SALMONOID IN A LIGHT GRADIENT

	Total illumination, no flow	Flow in Area A		
		Total illumination	Illumination in	
			Area A	Area B
<i>Chum fry controls</i>				
Number of groups	9	9	9	9
Observation periods*	17	18	18	18
Mean, fish in A	189	196	286	116
Mean, fish in B	171	164	74	244
t	0.954	1.216	10.812	5.069
P	0.35	0.26	<0.01	<0.01
<i>Coho fry controls</i>				
Number of groups	6	6	6	6
Observation periods*	10	10	12	12
Mean, fish in A	134	157	82	294
Mean, fish in B	226	203	278	66
t	2.558	1.058	8.232	7.980
P	0.02	0.3	<0.01	<0.01
<i>Coho smolt controls</i>				
Number of groups	6	6	6	6
Observation periods*	11	12	12	12
Mean, fish in A	137	203	101	267
Mean, fish in B	223	157	259	93
t	2.270	0.906	5.230	6.946
P	0.04	0.35	<0.01	<0.01
<i>Trout yearlings</i>				
Number of groups	6	6	6	6
Observation periods*	12	12	12	12
Mean, fish in A	147	198	107	276
Mean, fish in B	213	162	253	84
t	1.320	1.217	5.314	11.328
P	0.2	0.25	<0.01	<0.01

* Total number of positions plotted is in each case 360 times the number of observation periods. Total number of half-minute periods of observation is in each case 30 times number of observation periods.

TABLE II
EFFECT OF HORMONES ON THE RESPONSES OF CHUM SALMON FRY TO LIGHT

	No flow, uniform illumination	Flow in Area A		
		Uniform illumination	Illumination in Area A Area B	
<i>Control 12.5°-16.0° C.</i>				
Number of groups	9	9	9	9
Observation periods*	17	18	18	18
Mean, fish in A	189	196	286	116
Mean, fish in B	171	164	74	244
t	0.954	1.216	10.812	5.069
P	0.35	0.26	<0.01	<0.01
<i>Thyroxine 12.5°-16.0° C.</i>				
Number of groups	6	6	6	6
Observation periods*	8	9	9	9
Mean, fish in A	183	190	257	171
Mean, fish in B	177	170	103	189
t	0.276	0.280	7.839	0.661
P	>0.5	>0.5	<0.01	>0.5
<i>Testosterone 13.0°-16.0° C.</i>				
Number of groups	5	5	5	5
Observation periods*	9	10	10	9
Mean, fish in A	194	176	225	161
Mean, fish in B	166	184	135	199
t	1.268	0.277	2.304	1.064
P	0.22	>0.05	0.03	0.3
<i>Thiourea 13.0°-15.5° C.</i>				
Number of groups	5	5	5	5
Observation periods*	10	10	10	9
Mean, fish in A	187	200	293	151
Mean, fish in B	173	160	67	209
t	0.490	0.468	8.724	0.992
P	>0.5	>0.5	<0.01	0.35

* See footnote, Table I.

TABLE III

EFFECT OF HORMONES ON THE RESPONSES OF COHO SALMON FRY TO LIGHT

	No flow, uniform illumination	Flow in Area A		
		Uniform illumination	Illumination in	
			Area A	
<i>Control 14.0°-17.5° C.</i>				
Number of groups	6	6	6	6
Observation periods*	10	10	12	12
Mean, fish in A	134	157	82	294
Mean, fish in B	226	203	278	66
<i>t</i>	2.558	1.058	8.232	7.980
<i>P</i>	0.02	0.3	<0.01	<0.01
<i>Thyroxine 14.0°-17.5° C.</i>				
Number of groups	6	6	6	6
Observation periods*	12	12	12	12
Mean, fish in A	218	206	153	253
Mean, fish in B	142	154	207	107
<i>t</i>	3.526	1.747	1.906	5.825
<i>P</i>	<0.01	0.09	0.07	<0.01
<i>Testosterone 16.0°-17.0° C.</i>				
Number of groups	5	5	5	5
Observation periods*	8	10	10	10
Mean, fish in A	140	160	92	261
Mean, fish in B	220	200	258	99
<i>t</i>	2.152	1.372	7.304	6.820
<i>P</i>	0.05	0.2	<0.01	<0.01
<i>Thiourea 16.0°-17.0° C.</i>				
Number of groups	4	4	4	4
Observation periods*	8	8	8	8
Mean, fish in A	240	90	50	250
Mean, fish in B	120	270	310	110
<i>t</i>	5.796	7.722	16.224	2.926
<i>P</i>	<0.01	<0.01	<0.01	0.01

* See footnote, Table I.

A striking difference in responses of coho and chum salmon to light is evident. Coho fry show a marked tendency to concentrate in the darker end of the aquarium while the chum fry concentrate in the light. Coho smolt and trout yearlings were compared in the same gradient. Their reaction was

similar to that of the coho fry (Table I). These differences have not previously been measured and may be evidence of fundamental differences between aggregating or bottom-dwelling fish and schooling fish.

Hormone treatment does not change these basic responses to light although the intensity of the reactions is somewhat modified. Fig. 3 shows that both chum and coho fry treated with thiourea showed an over-all response almost identical with that of the control fish. On the other hand, thyroxine-treated and testosterone-treated fry of both species show a slight displacement toward the opposite end of the aquarium. This displacement is apparently related to the increased activity shown by these fish and is discussed in the next section.

Data presented for chum fry in Table II are interesting. Hormone-treated chum fry show one marked difference from the control fish. The lower values of "*t*" obtained when there was flow of water into the darkened end of the aquarium (last column, Table II) indicate that the rheotactic response was strong enough to partially counteract the effect of the light gradient. This is evidently due to the increased activity produced by the hormone treatment. The coho fry, however, do not show this difference (Table III). A contrast in the responses of coho and chum fry to currents has already been described (6). The low "*t*" values and probability levels shown in the two left columns of Table III indicate a departure of coho fry from uniform distribution under uniform light. Observation shows this to be due primarily to the territorial and nipping behavior of these fish (6). The very low "*P*" values for thiourea-treated fish may be related to the reduced activity and the increased tendency of these fish to aggregate.

Comparisons of Swimming Speeds

The findings are summarized in Table IV and Fig. 4. The temperatures in the hatchery varied from 13° to 14.5° C. during these experiments. In general, control and thyroxine-treated fish or thiourea-treated and testosterone-treated fish were observed on the same days.

TABLE IV

SWIMMING SPEEDS OF CHUM SALMON FRY EXPRESSED AS TIME IN SECONDS TO SWIM 25 CM.

	Number records	Mean sec.	Standard deviations	Fiducial limits $t_{.05} \bar{x}$
Controls	237	7.9	3.3	± 0.41
Thyroxine				
1 week	164	5.7	2.0	$\pm 0.32^*$
Thiourea				
1 week	108	8.8	6.3	± 1.23
2 week	69	15.6	9.5	$\pm 2.29^*$
Testosterone				
1 week	75	4.8	3.0	$\pm 0.70^*$
2 week	63	5.2	1.6	$\pm 0.40^*$

* Significantly different from controls.

It is evident that both thyroxine and testosterone increase the swimming speeds of chum fry schooling freely under uniform illumination. The anti-thyroid drug, thiourea, on the other hand, decreases the speed of the fish. During the first week of treatment, the thiourea fish do not differ significantly from the controls (95% level of significance) but the difference is marked during the second week of treatment.

The increased rate of swimming demonstrated here is in line with the qualitative observations made in the schooling and aggregating experiments.

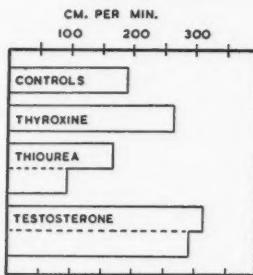


FIG. 4. Swimming speeds of chum salmon fry. Values above broken lines are for seven days' treatment; below broken line for 10-14 days' treatment; temperature range 13.0-14.5° C. Thyroxine-treated chum fry lose weight and show a mortality after about 10 days and fish treated longer than seven days with thyroxine have not been included.

Discussion

There is ample circumstantial evidence for an intimate relationship between the activity of the endocrine glands and changes in behavior of salmon associated with their migrations from one habitat to another. The seaward migration of juvenile salmon is often associated with a hyperactive thyroid gland and although this does not seem to be a primary mechanism controlling their migration, it may be a contributing factor (6, 8). Changes in thyroid activity (2) as well as gonad development are associated with the movement of mature salmon from the ocean into fresh waters. New patterns of behavior appear at sexual maturity when the fish occupy the spawning beds. In spite of this evidence suggestive of endocrine regulation of salmon behavior there is little experimental work to show how this regulation is effected (5). Before discussing the significance of the data recorded here it is important to determine whether hormone levels were altered in the fish by the methods used in these experiments.

A number of synthetic gonad hormones have been shown to produce changes in secondary sexual characters and behavior of the Poeciliidae when added to the aquarium water or the food or when injected (5). Immersion of *Platypoecilus* (females or castrated males) in solutions of methyl testosterone, the preparation used in the present experiments, induces prompt changes in the rate of gonopod growth (3). The increased activity shown by juvenile Pacific salmon immersed in methyl testosterone is evidence for the efficacy of this

treatment. Stanley and Tescher (15) report a 400% increase in the activity of goldfish (*Carassius auratus*) fed on testicular material; Hasler and Meyer (4) associate increased oxygen consumption with gonad stimulation in goldfish; and Raffy and Fontaine (14) recorded an elevation in oxygen consumption of the male guppy at sexual maturity. Thus, there is evidence in the literature that methyl testosterone will enter the tissues of fish immersed in solutions and that this hormone might be expected to bring about the increased rate of swimming observed in our experiments.

Immersion in synthetic thyroxine sodium produces prompt changes in the metabolism of salmon fry. Deposition of guanine is increased in both coho and chum fry (5). In some groups definite differences are apparent within seven days (7). Immersion of goldfish in synthetic thyroxine likewise brings about changes in metabolism (7). It is safe to conclude that the metabolism of the salmon fry used in the present study was modified within the one-week minimum period of treatment with thyroxine.

Thiourea solutions have been shown to inhibit the rate of growth and differentiation of young salmon immersed in them (8). In the present study little change in activity was noted during the first week although the fish are more easily startled by floating particles and surface ripples in the circular rheotaxis tubs. During the second week the reactions, particularly the rate of swimming, are slower.

Thiourea is known to stimulate the epithelial cells of the thyroid gland but to prevent the formation of organic iodine compounds responsible for the specific effects of the thyroid hormone (1, 10). Within one week, fish treated with thiourea show a significant change in thyroid activity (11). It seems probable that a prompt release of stored colloid, resulting from immersion in thiourea, will stimulate the salmon fry and cause increased swimming for a few days. The effect of thyroid inhibition will not be immediately manifest and the slowing of the swimming does not become marked until the second week (Table IV). The thiourea experiments are in agreement with the thyroxine experiments and in accordance with the present information on the action of the antithyroid drugs.

Salmon treated with methyl testosterone show several variations in activity. Chum fry school less intensively (Fig. 1) and swim more rapidly (Fig. 4). The first change may be dependent upon the second. It is possible that the increased activity will result in a more loosely knit school. However, the data show quite definitely that startling fish or disturbing them usually results in a more closely knit school (Figs. 1 and 2). For this reason, it is suggested that the less intense schooling of the treated chum fry is due to a particular effect of the hormone on the schooling reaction. Less intense schooling in sexually mature fish has been recorded (13). Although differences in the intensity of reactions seem evident, no new patterns of behavior were observed either in coho or chum salmon. This is perhaps surprising since reproductive behavior has frequently been induced in fish by gonad stimulation (5). Size cannot be alone responsible since male Atlantic salmon parr (*Salmo salar*)

may become sexually mature and show characteristic behavior in relation to adult female salmon (9). It seems probable that either hormone treatment was not sufficiently prolonged or additional factors (pituitary hormone, appropriate releasers from female fish) are required to elicit them.

Coho fry treated with methyl testosterone show less change in activity than chum fry (Figs. 1 and 2). This is thought to be due to their marked territorial behavior and failure to form definite groups under normal conditions (6). Minor changes in photoresponse evident in both chum and coho fry treated with testosterone may be due to changes in rate of swimming or may reflect a change in light sensitivity. Present data do not permit any conclusion as to the significance of this difference.

The findings for fish treated with thyroxine are essentially the same as those for fish treated with testosterone. Treatment with thiourea produces responses which are, in general, opposite to those produced by thyroxine. The data indicate that thyroid stimulation may be expected to increase the rate of swimming and general activity of fish, and to lessen the intensity of schooling and aggregating. New patterns of behavior do not seem to be evident in thyroxine-treated fish. If the thyroid gland plays a part in the migration of salmon, its action would seem to be one of increasing the general activity of the fish and controlling the osmotic balance (8).

In general, it is concluded that immersion of the salmon fry in solutions of thyroxine, testosterone, and thiourea changed the level of hormones within the fish. Thyroid and male gonad hormones increase the general activity but do not change the basic behavior patterns of these juvenile fish with respect to schooling, aggregating, or photoresponse.

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THE POPULATION AND ACTIVITY OF ADULT FEMALE BLACK FLIES IN THE VICINITY OF A STREAM IN ALGONQUIN PARK, ONTARIO¹

BY DOUGLAS M. DAVIES²

Abstract

The population of females of *Simulium venustum* Say on the wing was measured by hand-netting. The population varied annually and seasonally coincident with the number and longevity of immigrant and emergent flies. Longevity in turn was probably related to the rainfall.

Activity was divided into flying, attraction, landing, and biting, the first two measured by hand-netting and the last two by counts on a unit area of human skin in the shade. Flying usually varied diurnally, a large peak in the evening and a small one in the morning. Attraction varied with none of the meteorological factors measured. Biting and flying increased and landing decreased with rapidly changing, especially falling, pressure. Other factors influenced flying and landing, but not biting, when the area was in the shade. Flying was greatest between 60–80° F., at low but not zero saturation deficiencies, in light winds, and in zero to low rates of evaporation. Landing on the host was least below 55° F. and at 65–75° F., at zero and intermediate saturation deficiencies, and at moderately high rates of evaporation. These factors affected the flies landing on the host directly, and indirectly by curtailing the flying that brought flies to the host in the first place. In the direct sunlight landing decreased to one-half and biting to one-quarter of that in the shade.

Part I. Variation in the Size of the Population

A study of the seasonal variation of the black fly population in the vicinity of Costello creek, Algonquin Park, Ont., was made in 1946 and 1947. This was carried out simultaneously with research on the population of black flies emerging from Costello creek (4).

METHODS

Adult black flies were collected during the spring and summer at a station 25 yd. from the stream. To make the collection, the observer approached the station rapidly and began sweeping immediately through a 6 ft. arc with an insect net of 11½ in. diameter at the mouth. In the first method of sweeping, used only in 1946, 40 horizontal sweeps at shoulder height were made with the net at arm's length, pausing two seconds after every two sweeps. In 1946 and 1947 a second method was employed in which 40 horizontal sweeps close to the body were made, five at head level alternating with five at ankle level. The sweeps were made at approximately one second intervals with a 10 sec. pause between each 10 sweeps.

For each day the mean number of flies per sweep collection was calculated. A five-day moving average of the daily means (an average of five daily means, including the two days preceding and following the figure for the day) was

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used to reduce variations caused by the influence of the observer or meteorological factors, that might obscure the seasonal pattern. The square roots of the numbers were used to make the variance more nearly constant.

Other sweep collections at locations within 1000 yd. of the stream were used to determine the length of the season that different species of black flies were on the wing.

Relative humidity and atmospheric temperature were recorded by a Negretti and Zambra hygrograph (Model 5669-72) and a Tyco thermograph (Model 2350) which were kept in a thermometer screen at the site and were standardized periodically with a Taylor sling psychrometer (Model 1322). Rainfall was measured with a rain gauge.

RESULTS

Sweep collections, which were made at the station from June 14 to July 12, yielded six times as many black flies in 1947 as in 1946 (Table I). Coincident with the scarcity of flies in the field in 1946 was the smaller emergence from Costello creek being a third of that in 1947 (4). In addition there was a light rainfall during June and July in 1946 as compared to that in 1947 (Table II) and the average temperature (Fig. 1) and saturation deficiency of the air were higher. It seems probable therefore that the longevity of the flies after emergence was much shorter in 1946. This was shown also by the fact that over the first 400 ft. of rapids in Costello creek, only about 500 females

TABLE I

THE NUMBER OF FEMALES IN SWEEP COLLECTIONS OF THE SECOND METHOD AT THE STATION FROM JUNE 14 TO JULY 12 IN 1946 AND 1947 AND THE NUMBER EMERGING FROM COSTELLO CREEK FROM JUNE 1 TO JULY 12

Year	No. of sweep collections	Total No. of flies	No. flies per collection	No. emerged per sq. yd.
1946	47	358(333)*	7.6(7.1)*	(5416)*
1947	76	3244(3037)*	42.7(40.0)*	(19,629)*

* Number of females of *S. venustum* in brackets.

TABLE II

COMPARISON OF THE RAINFALL IN 1946 AND 1947 IN INCHES

Year	May 4-31	June	July	August	Total
1946	2.9*	1.1	1.3	2.0	7.3
1947	3.5	2.6	4.1	0.8	11.0

* First few observations without rain gauge.

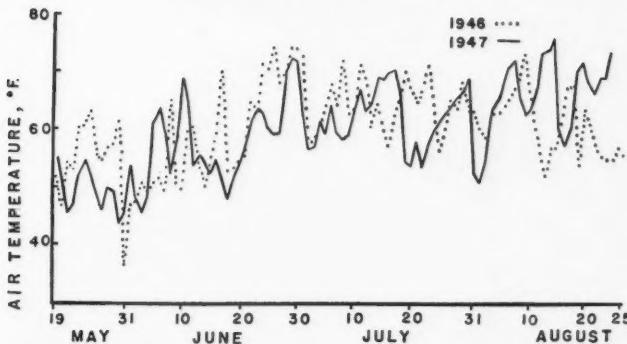


FIG. 1. Seasonal variation in mean daily air temperature (an average of the daily maximum and minimum) for the years 1946 and 1947 in the thermometer screen at Costello creek.

of *S. venustum* Say were observed ovipositing in 1946 as compared to 25,000 during the same period in 1947. Observations indicated that oviposition does not occur until two to three weeks after emergence (4). Consequently it appears that few flies lived for this length of time in either year, particularly in 1946.

Females of *Simulium venustum* Say comprised about 90% of the flies in the sweep collections so that the seasonal variation of this species only is discussed in detail. Females were netted at the station as early as May 17 in 1946 but not until May 29 in 1947, which is indicative of the later season.

There was a peak in numbers on June 4, 1946, before the standard method of sweeping was developed but probably the greatest number of flies in 1946 was netted at the station on June 12 (Fig. 2a). The first peak was caused by flies immigrating into the area from other streams because it occurred before emergence began in Costello creek (4). The second peak was probably mainly the result of an immigration of flies also, because it preceded by three weeks the peak of emergence in Costello creek. Thereafter there were few flies in the field in 1946 even when there were 2000 flies emerging daily from a square yard of Costello creek. Coincident with the rapid decrease in the number of *S. venustum* at the station was the increasing dryness of the ground as a result of scant rainfall (Table II) and in addition the increased temperature (Fig. 1) and saturation deficiency of the air.

The first peak in numbers in 1947 (Fig. 2b) was probably in large part the result of flies immigrating to the area from other streams as it was reached before the first peak of emergence in Costello creek (4, Fig. 5). Then fewer flies were taken for a period and at the same time the mean daily atmospheric temperature decreased. The population at the station increased before the temperature began to rise; apparently the result of the increasing number of emerging flies. The second peak in numbers at the station on June 23 coincided with the largest peak in emergence of *S. venustum* from Costello creek, following which there was relatively rapid decrease in the number of flies

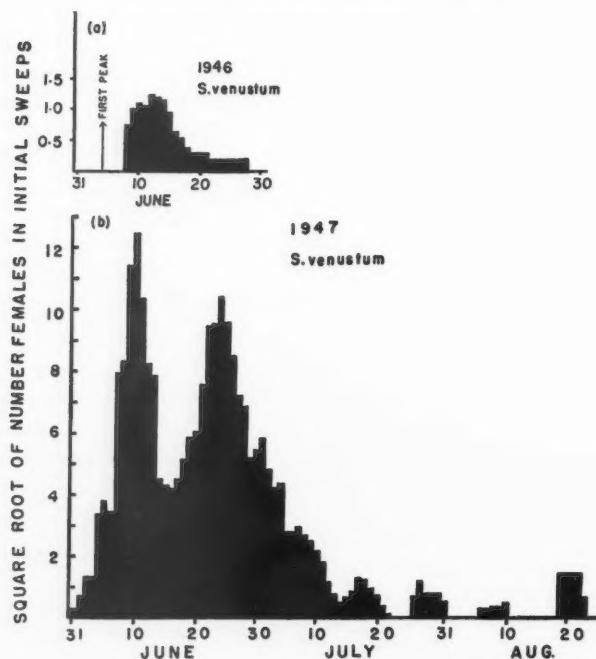


FIG. 2. Seasonal variation in the number of females of *S. venustum* netted at a station 25 yd. from Costello creek and expressed as the square root of a five-day moving average of the daily mean number per sweep collection. In June 1947 the second method of sweeping was used which yielded, on the average, four times as many flies as did the first method of sweeping used in 1946. The ordinate scales are adjusted with this in mind.

netted, although a small upsurge in the number occurred on July 17 coinciding with the peak in emergence on the same date. The decrease was associated, as in 1946, with a gradual drying of the ground (Table II) and with higher temperatures (Fig. 1).

Other species, in addition to *S. venustum*, were taken in sweep collections but in smaller numbers. The seasonal span of the occurrence of different species in the vicinity of Costello creek during 1946 is compared with that during 1947 (Fig. 3). All species, except *S. venustum*, were taken in sweep collections until a later date in 1947 than in 1946. Females of *Prosimulium hirtipes* (Fries) were netted at Costello before emergence began from Costello creek and came to the vicinity presumably from other streams in which this species was observed emerging at an early date. Thirty females of *Prosimulium decemarticulatum* Twinn were taken in sweep collections in 1947 although none appeared in emergence collections and only one pupa occurred in collections of immature stages. On the other hand few specimens of *Simulium vittatum* Zetterstedt or *Simulium decorum* Walker and none of *Cnephia dacotense* (Dyar and Shannon) were obtained by netting, even though they were

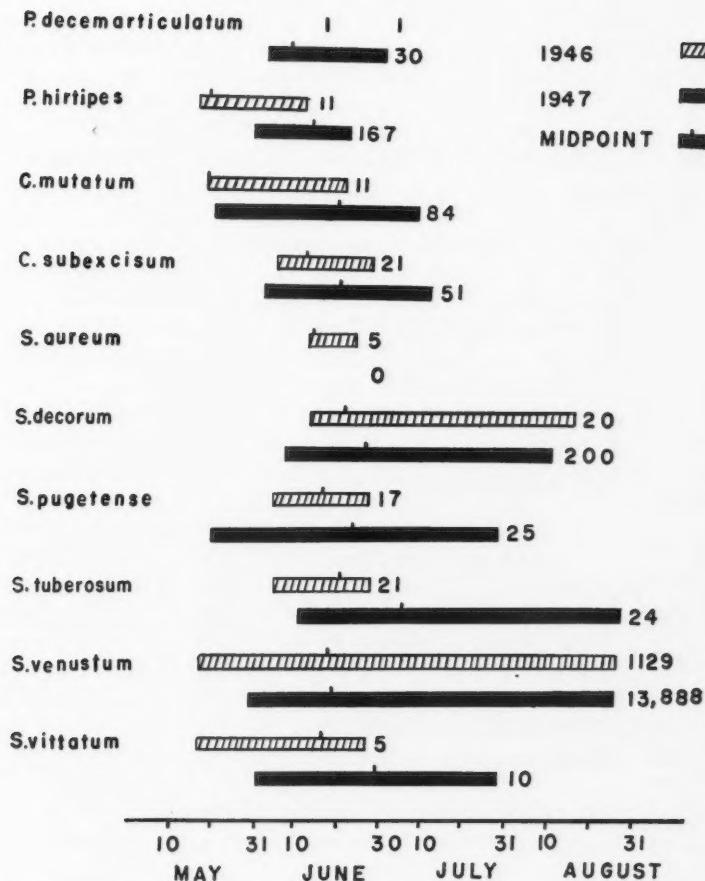


FIG. 3. The span and midpoint of occurrence of females of different species in 1946 and 1947 as gauged by sweep collections taken within 1000 yd. of Costello creek. The total number of individuals netted is indicated at the end of each bar.

numerous in the emergence collections in 1947. In the case of *C. dacotense* this is not surprising as mating and oviposition occur shortly after emergence, with little flying beyond the immediate bank of the stream.

Few males were taken in sweep collections, single males of *Cnephia mutatum* (Malloch), *S. vittatum*, and *S. venustum* in 1946 and one of *S. venustum* in 1947.

Thus the sweep collections of 1946 and 1947 in the vicinity of Costello creek indicated that females of *S. venustum* made up about 90% of the population of flying individuals. This population was small in 1946, coincident with a small emergence from the stream and with a scant rainfall. The population of flying individuals varied seasonally, being largest in June and decreasing rapidly thereafter. The peaks were influenced early in the season more by

immigrant flies but later they coincided with peaks in emergence from Costello creek itself. The population of flying individuals in 1946 (mainly immigrant flies) decreased rapidly after mid-June coincident with a drying of the ground, in spite of the many flies emerging from Costello creek.

Part II. Variation in Activity with Environmental Factors

INTRODUCTION

In recent years there has been an increasing amount of research devoted to the influence of environmental factors on the activity of insects. A comprehensive summary of the earlier work on insects and climate was published by Uvarov (15) but contained little about black flies. Underhill (12, 13) presented data on the variation of the feeding of black flies on turkeys with changes in meteorological conditions.

The present study in Algonquin Park, Ont., was undertaken to obtain more detailed knowledge of the activities of black flies and the variation of their activities with environmental factors, especially with meteorological factors. The number of active female black flies in an area at a given time depends on factors affecting the size of the adult population in that area during the season as well as on the immediate environmental factors influencing activity. Therefore, most of the studies of activity were made simultaneously with those on populations of black flies (4). Four activities under field conditions were studied, namely flying, attraction, landing, and biting. The number of flies participating in each activity was related to the size of the population from which it was derived. Thus the population of flying individuals is the active portion of the total population in the area; those flies attracted to the observer, but still flying, are derived from the population of flying individuals; those flies landing on the observer are derived from the population of those attracted; and those flies biting the observer are derived from those that landed. These activities were compared to variations in environmental factors that were observed at the same time.

METHODS

Measurements of Environmental Factors

Changes in atmospheric pressure were recorded in the laboratory with a Short and Mason microbarograph. The barograph was standardized by means of a Fortin mercury barometer.

In 1946 and 1947 a thermometer screen was located 50 yd. from Costello creek in an open field three feet above the ground. In it were housed a thermograph and a hygrograph that continuously recorded temperature and relative humidity respectively (see Part I).

In 1947 other observations of environmental factors were taken at the immediate site where counts of flies flying, landing, and biting were made. Subsoil temperatures were taken with a Kimble thermometer (model 2600), the bulb being inserted obliquely just below the surface of the soil in the shade.

Wind speeds of 100-3000 ft. per min. at two feet from the ground were measured with a Biram's type anemometer (Tyco model 3132) that was turned into the wind by hand. Several readings were averaged if the wind was gusty. Lower wind speeds at the same level were measured by two Kata thermometers (95 to 100° F. and 65 to 70° F.). In order to calculate the wind speed from the Kata readings, the atmospheric temperature at one foot from the ground was recorded with the dry bulb of a Taylor dry and wet bulb hygrometer. The amount of evaporation in unit time was measured at one foot from the ground in the shade with a Negretti and Zambra Piche atmometer. The light intensity was measured with a Weston Master exposure meter (model 715), directed at right angles to an unshaded white enamelled plate ($5\frac{1}{4} \times 2\frac{3}{4}$ in.) two inches away. The readings from 200 to 0 were converted to foot candles.

In 1948 the thermometer screen containing the thermograph and hygrograph was located in an open clearing 100 yd. from the "sweep" station on the shore of lake Sasajewun. Also in the screen was a Piche atmometer from which evaporation was noted hourly. At the "sweep" station measurements of the subsoil temperature, immediate wind speed and direction, and also the number of feet of wind passing the station per hour were observed.

Procedure in Measuring the Activities of Black Flies

The female population on the wing at a single "sweep" station is a sample of the total female population of one small area. This population of flying individuals at the "sweep" station was sampled by the initial sweep collection that is described as the second method of sweeping in Part I. The sweep collections were made by the same worker wearing the same outer clothing throughout the season to minimize any variation that might be caused by different observers or color and amount of clothing.

Initial sweep collections were made (1) hourly from 8:00 a.m. to 8:30 p.m. on June 24, 1946, at a station 25 yd. southwest of Costello creek; (2) once or more on almost every day from May 31 to Aug. 27, 1947, at the same station southwest of Costello creek; and (3) hourly during daylight hours on June 14, 15, 19, 21, 25, and 26, 1948, at Lake Sasajewun, Algonquin Park, 10 miles west of Costello creek.

In 1947, after each initial sweep collection was made, the meteorological readings, other than those taken by recording instruments, were noted. Following this the observer sat and counted simultaneously, during a two-minute period, the numbers of flies landing and biting on an area of skin, 3×3 in., that was outlined in ink on his abdomen. The observer sat with his back to the direct sunlight and as much as possible out of the wind. Usually several two-minute counts were made, one after the other. After each count, a sample of the flies that were still biting was taken with a suction apparatus (Fig. 4) and preserved in 95% ethanol. A second sweep collection (40 sweeps) was taken after the series of counts, followed by another reading of the meteorological instruments.

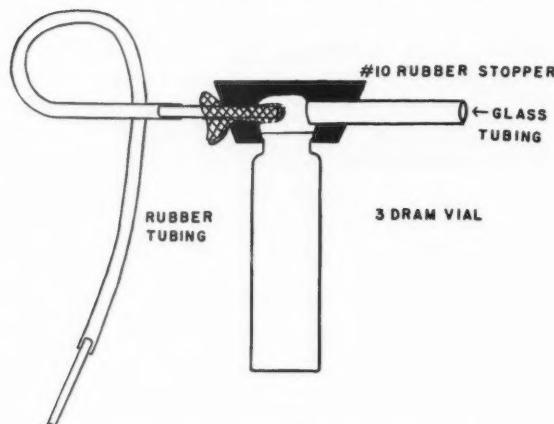


FIG. 4. Suction apparatus for collecting black flies after they landed. The 3-dr. vial is detachable.

Early as well as late in the season, when flies were not as abundant, the landing and biting counts lasted from 5–17 min. and were made sometimes on a larger area. The data obtained from all counts were converted to a uniform basis, viz., the number of flies landing (or biting) in 10 min., on an area of nine square inches.

In 1948 after each initial sweep collection was made, the meteorological readings from instruments at the site were noted. A second sweep collection (40 sweeps) was made approximately five minutes after the initial sweep collection, after which the atmometer in the screen was read.

RESULTS

The activities of adult female black flies were divided into four categories: flying, attraction, landing, and biting.

The flying activity was measured by the initial sweep collections in 1946, 1947, and 1948. The number in these collections, in addition to being an indication of flying activity, reflects the seasonal variation in the size of the total population in the vicinity (see Part I). The numbers in the collections of 1947 were corrected for daily and individual variation so that the numbers would more nearly represent a standard proportion of the total population. The amount of the correction in each case or the difference of the number in each collection from the mean seasonal line (Fig. 2b), whether positive or negative, should be more nearly representative of fluctuations in flying activity than is the number in the original sweep collection. In some cases, however, the data from sweep collections, both corrected and uncorrected for seasonal variation, were used.

The attraction of the flies on the wing to the host was measured in 1948 by the ratio of the second sweep collection to the initial sweep collection.

The landing and biting activities were studied in 1947. The landing activity is expressed by the ratio of the number landing to the number in the nearest sweep collection and the biting activity by the ratio of the number biting to the number landing.

The variation of each of these activities was studied with changes in environmental factors, particularly meteorological factors.

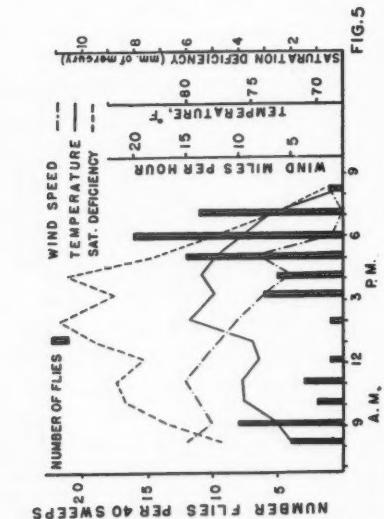
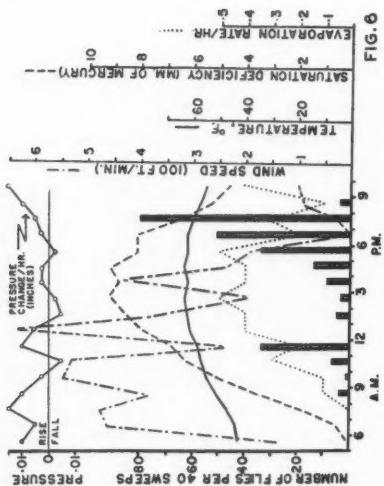
Variation in Flying Activity During a 24 hr. Period

Qualitative observations indicated that black flies are frequently more abundant on the wing in the early evening than at other times. By making hourly sweep collections in 1946 and 1948 the daily rhythm in flying was studied more quantitatively and in relation to meteorological conditions.

The species composition of the hourly sweep collections made on June 24, 1946, was as follows: *S. venustum* Say (84.3%), *S. decorum* Walker (7.4%), *C. subexcisum* (Edwards) (3.3%), *S. tuberosum* Lundstroem (2.5%), *S. pugetense* (D. and S.) (1.6%), and *S. vittatum* Zett. (0.8%). In 1948 the hourly initial sweep collections were made up of the following species: *S. venustum* (82%), *S. pugetense* (15.8%), *S. tuberosum* (1.8%), and *C. subexcisum* (0.4%). Thus the study of hourly activity is confined to *S. venustum* because it was the only species netted in numbers during the experiments.

In sweep collections taken on June 24, 1946, there was a large peak in numbers at 6:00 p.m. and a smaller one at 9:00 a.m. (Fig. 5). On June 24, as on several days preceding it, there was a well-marked weather pattern, the middle of the day being hot with a high saturation deficiency and considerable cooling occurring at night accompanied by a lower saturation deficiency. The increase in the number of flies after the first collection coincided with a rising atmospheric temperature but after 9:00 a.m. the number decreased coinciding with an increase in the saturation deficiency (Fig. 5). The number began to increase after 2:00 p.m., the increase accompanying a decreasing saturation deficiency, temperature, and wind speed. After 7:00 p.m. there was a marked decrease in number, coincident with a rapid fall in the temperature. The number of flies on the wing varied with saturation deficiency and wind speed but temperature also exerted some control. There appeared to be no relation between the number of flies on the wing and the absolute pressure.

Hourly sweep collections were made on seven days in 1948 but only three, representing different activity patterns, are presented (Figs. 6-8). The collections on June 15 and 19 show a rhythm similar to that found on June 24, 1946. The number of flies on the wing was compared with the evaporation rate, a resultant of several factors including the saturation deficiency, wind speed and the temperature of the air. There were few flies in sweep collections from about 11:00 a.m. to 7:00 p.m. During this period of the day there was a high rate of evaporation with a distinct peak during midafternoon (Figs. 6 and 7). On June 25, 1948, on the other hand, the evaporation rate remained



[Figs. 5-8 to be viewed broadside.]

FIG. 5 (Top left). Histogram of the variation in number of females of *S. venustum* taken in sweep collections of the second method on June 24, 1946, at Costello creek and the variation of certain meteorological factors measured at the same times.

FIG. 6 (Top right). As in Fig. 5 but collections taken on June 15, 1948, at Lake Sasajewun.

FIG. 7 (Bottom left). As in Fig. 5 but collections taken on June 19, 1948, at Lake Sasajewun. Wind speed expressed as 1000's ft. per hr. was calculated from the total number of feet of wind passing site between each sweep collection.

FIG. 8 (Bottom right). As in Fig. 5 but collections taken on June 25, 1948, at Lake Sasajewun.

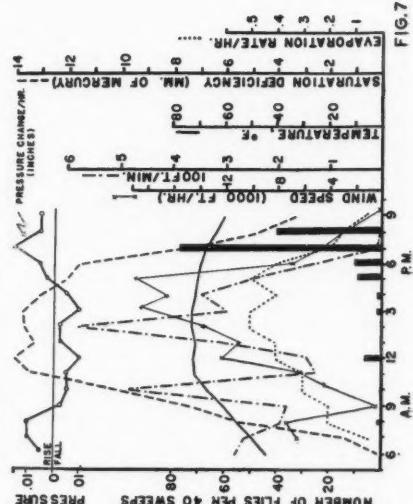
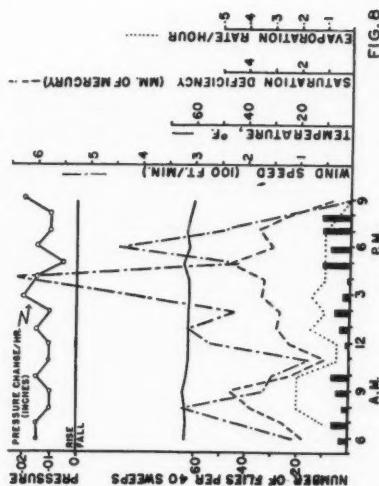
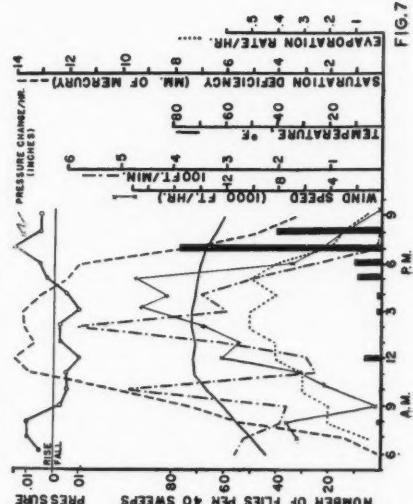


FIG. 7



low for the whole day and the flying activity remained at a more even level throughout as gauged by the sweep collections (Fig. 8). The number of flies on the wing varied closely with rate of evaporation.

Temperature appears to be of primary importance in determining how early flies will be on the wing in the morning. The higher the early morning sub-soil or atmospheric temperature the sooner were the flies active (Fig. 8). The effect of warm temperature was reduced or nullified and flying was delayed if the early morning was windy. The morning peak in activity was usually small and of short duration because the saturation deficiency and the wind speed increased rapidly, raising the rate of evaporation. The rate of evaporation varies both with the saturation deficiency and with the number of feet of wind passing a certain point in an hour. The influence of the saturation deficiency and the average wind speed per hour on the flying activity may operate through its effect on the rate of water loss from the insect. However, the immediate wind speed at time of sweeping was probably important both in its influence on the immediate water loss from the insect and through mechanical action, and was reflected in the number of flies in the collections. As the saturation deficiency and wind speed decreased towards the end of the day, the number of flies on the wing increased (Figs. 6 and 7) until the light waned, although the decreasing temperature in the evening may also have reduced flying. The flying activity, on the days studied, did not appear to vary with the rate of change of atmospheric pressure although the fluctuations in pressure were not great on these days (Figs. 6 to 8). Flies were not obtained in the sweep collections after dark, but during most of the day, when the light intensity was over 300 ft-c., the number on the wing appeared to vary little with light intensity.

The rhythm in flying activity varied in large part with periodic variation in meteorological factors and when these environmental factors showed non-periodic variation, as in Fig. 8, or in the case of a storm, the rhythm of activity was reduced or lost. Only extreme conditions of some factors, such as light, influenced the activity, whereas smaller changes in others, such as saturation deficiency and wind speed, were reflected in the activity of the flies.

Variation in Flying Activity with Changes in Environmental Factors

The flying activity, as represented by the number of flies in the sweep collections of 1947, both corrected and uncorrected for seasonal variation, was studied with changes in individual environmental factors, mainly meteorological factors. The species investigated was *S. venustum* which formed over 90% of the black flies netted.

Flying activity was studied under different conditions of atmospheric pressure. The flying activity, as indicated by the uncorrected number of flies in the sweep collections, varied with changes in pressure in the half hour or hour preceding each collection (Figs. 9 and 10). Few flies were taken when the pressure was changing slightly but many when the rate of change increased, especially with falling pressures. Similar variations with pressure change

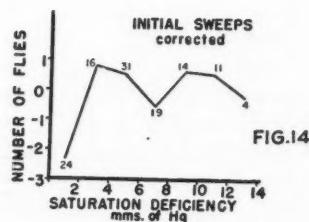
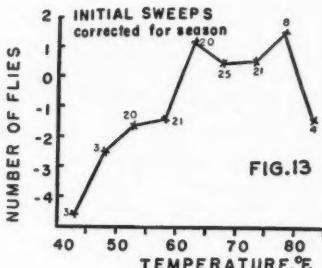
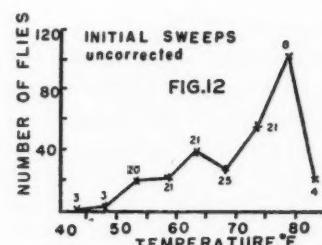
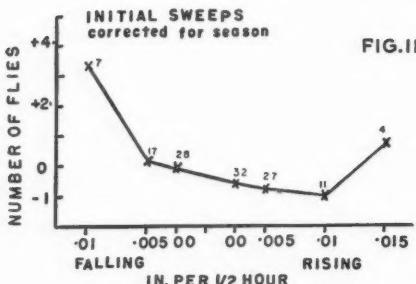
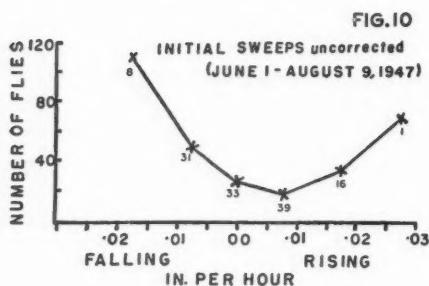
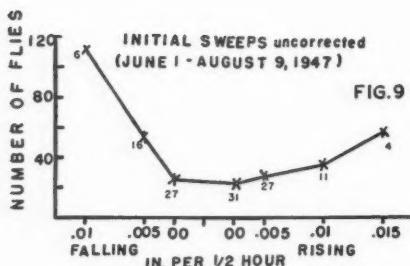


FIG. 9. Variations in flying activity (uncorrected) of females of *S. venustum* with the change in atmospheric pressure during the half hour preceding each sweep collection. A change of less than 0.005 in. per half hour is designated as "00". The number of observations on which each point is based appears beside it.

FIG. 10. Variation in flying activity (uncorrected) of females of *S. venustum* with the change in atmospheric pressure during the hour preceding each sweep collection.

FIG. 11. Variation in flying activity (corrected) of females of *S. venustum* with the change in atmospheric pressure during the half hour preceding each sweep collection.

FIG. 12. Variation in flying activity (uncorrected) of females of *S. venustum* with temperature.

FIG. 13. Variation in flying activity (corrected) of females of *S. venustum* with temperature.

FIG. 14. Variation in flying activity (corrected) of females of *S. venustum* with saturation deficiency.

were observed with the corrected data but the flying activity was less with rising pressure changes (Fig. 11). The corrected or uncorrected number in the sweep collections did not vary with the total change in pressure during the preceding three hours nor with absolute pressure. There were more flies in the sweep collections taken in gusty winds than in nongusty winds although the difference was not statistically significant. This may be also an effect of pressure because gusty winds, changes in wind speed amounting to 50 to 250 ft. per min., are rapid fluctuations in pressure.

The variation of flying activity with atmospheric temperature was analyzed. The number of flies in the sweep collections increased with rising temperature up to 80° F. Most were taken at 75–80° F. in the uncorrected collections (Fig. 12). When the collections were corrected however, high numbers were obtained from 60–80° F. but activity decreased rapidly below 50° F. (Fig. 13). Above 80° F. the numbers decreased, based on four observations only. These results may be the direct effect of temperature on the fly or the influence of other meteorological factors interdependent with temperature. It was observed also that half as many flies were on the wing when the subsoil temperature was below 50° F. as when it was above.

The number of flies in the corrected and uncorrected sweep collections when plotted against saturation deficiency (Fig. 14) gave two peaks, one at 3 mm. and the other at 9 mm. of mercury. The number, when plotted against relative humidity showed peaks at 80% and 40% respectively. The peak in the drier air, however, was based on fewer observations.

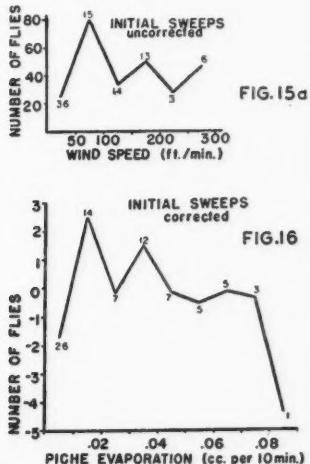


FIG. 15a

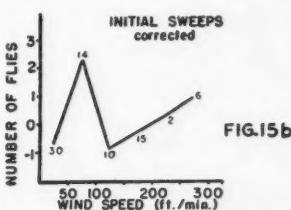


FIG. 15b

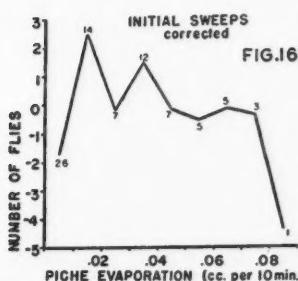


FIG. 16

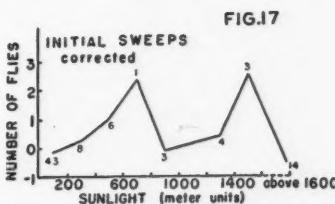


FIG. 17

FIG. 15. Variation of flying activity of females of *S. venustum* with wind speeds. (a) uncorrected (b) corrected.

FIG. 16. Variation in flying activity (corrected) of females of *S. venustum* with evaporation rate as measured by a Picche atmometer.

FIG. 17. Variation in flying activity (corrected) of females of *S. venustum* with light intensities as measured by an exposure meter.

The wind may affect the flying activity directly by blowing the flies in or out of the area or indirectly through the rate of evaporation. The largest number, corrected and uncorrected, were netted in wind speeds between 50–100 ft. per min. (Fig. 15). More flies were present with winds from an easterly to southerly direction, that is from the stream and lake margins.

The number of flies on the wing was observed at different rates of evaporation. The greatest number, corrected and uncorrected, was taken at evaporation rates of 0.01–0.02 units per 10 min. on a Piche atmometer (Fig. 16). The reason for more flies being on the wing at low but not zero evaporation rates may be partly explained by the fact that low evaporation rates frequently occurred at the beginning and end of the day when low temperatures occurred. At these times low temperature may curtail activity. Thus when only temperatures from 60–80° F. are considered, more flies were collected at evaporation rates of 0–0.01 units than at higher values.

The change in the number in the sweep collections was studied with changes in light intensity. In the corrected or uncorrected collections two peaks occurred at 500–700 units and at 1400–1600 units, both apexes based on few observations (Fig. 17). In general the number of flies on the wing appeared to increase with light but above 1600 units the number decreased again.

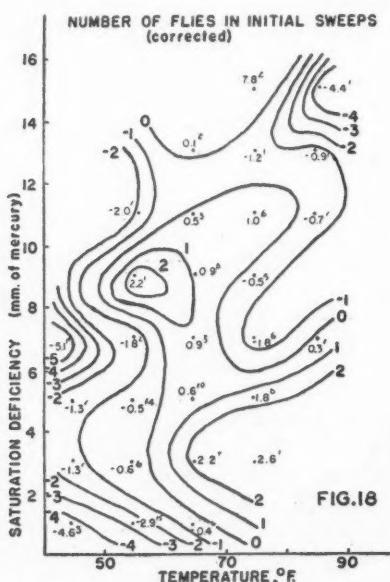


FIG. 18

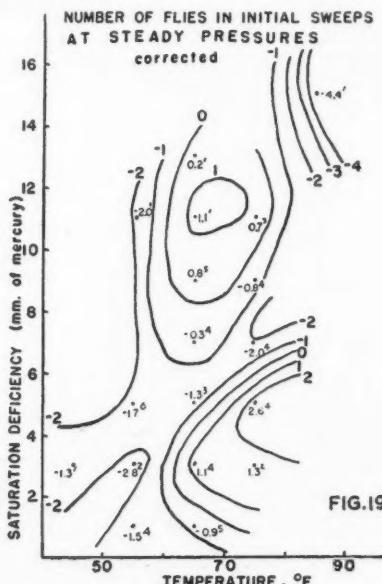


FIG. 19

FIG. 18. A plan of three-dimensional graph which represents, by isopleths, the flying activity (corrected) varying with air temperature and saturation deficiency.

NOTE: The fine numerals at the points are actual averages and the smallest numerals indicate the number of observations on which each average is based.

FIG. 19. A plan of a three-dimensional graph which represents, by isopleths, the flying activity (corrected) at steady atmospheric pressures varying with temperature and saturation deficiency.

Although usually fewest flies were on the wing below 100 units (275 ft-c.) sometimes many flies were netted and observed landing and biting on humans at light intensities as low as one foot candle. Black flies are seldom found on the wing after dusk. However, flies brought indoors and released continued to seek the human host and bite under conditions of artificial illumination.

The variation of flying activity was analyzed also with simultaneous changes in two factors. The data are plotted as the plan of a three dimensional graph in which each of the two meteorological factors is an axis and the numbers of flies are represented by isopleths. The number on the wing was compared with variations in the temperature and moisture content of the air. The greatest number of flies (corrected for seasonal variation) was netted at temperatures from 65–75° F. and at saturation deficiencies from 2–4 mm. of mercury (Fig. 18) or at relative humidities from 70–80%, but when the temperature dropped to 55° F. more flies were flying at saturation deficiencies of 8–10 mm. of mercury. If collections taken only at steady pressures

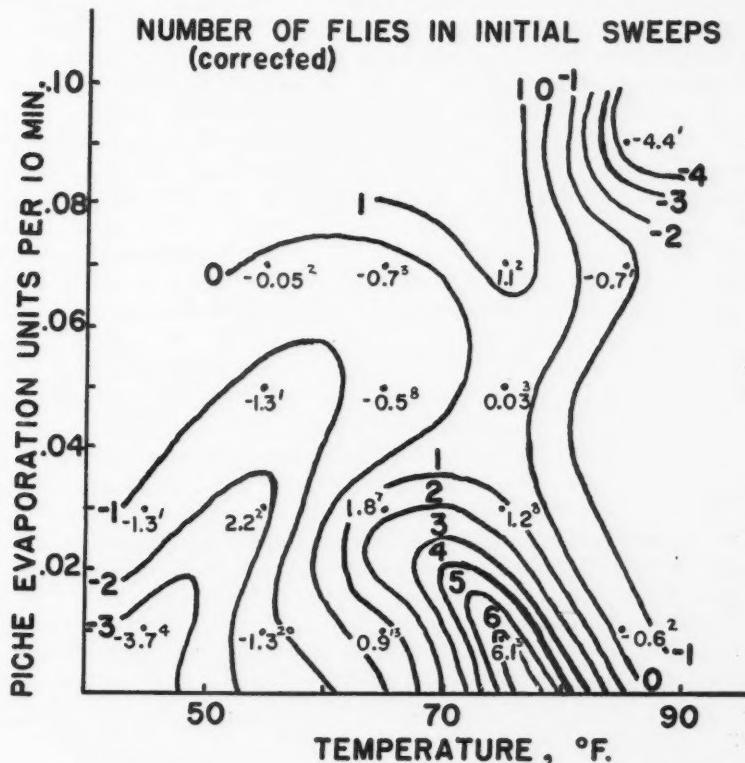


FIG. 20. A plan of a three-dimensional graph which represents by isopleths the flying activity (corrected) of females of *S. venustum* varying with temperature and rate of evaporation.

(pressure changes of less than 0.005 in. per half hour) are considered, the greatest number of flies was found at temperatures of 70–80° F. and at saturation deficiencies of 3–5 mm. of mercury (Fig. 19) or at relative humidities of 70–90%. The fewest flies were taken at temperatures below 60° F., but one collection at temperature above 80° F. and at a saturation deficiency of 14–15 mm. of mercury contained even fewer flies.

Variation in flying activity was studied also with simultaneous changes in atmospheric temperature and rate of evaporation. Most flies were netted at temperatures from 70–80° F. and at rates of evaporation from 0–0.02 units per 10 min. on the Piche atmometer (Fig. 20).

Variation in Attraction with Changes in Meteorological Factors

The attraction of black flies to the human is expressed by the ratio of the second to the initial sweep collection, the two sweep collections being made approximately five minutes apart. When the number of flies taken in each initial collection is plotted on a graph against the number in each second collection there is a good straight line correlation. That is the ratio remains fairly constant and there appears to be little variation which might be related to meteorological conditions. When the ratios found in each of the tests were compared with different weather factors measured at the same time, the attraction could not be shown to vary significantly with any meteorological factor. Therefore, although black flies are definitely attracted to the human, the attraction does not appear to be greatly influenced by meteorological conditions.

In other tests it was found that increasing the time between sweeps to 30 min. did not change the ratio greatly. It may be that most of the flies attracted to fly around the host are influenced within the first five minutes.

Variation in Landing Activity with Changes in Meteorological Factors

The actual number in initial and final sweep collections represents a sample of the flying population from which are derived the landing and biting females. The number of females landing on the host is not by itself truly an activity and should be related to the number flying. The first and last landing counts were divided by the number in the initial and final sweep collections respectively and intermediate landing counts by interpolated sweep numbers. The square roots of the numbers were used to maintain a more nearly constant variance.

It was shown by statistical test that the above "landing/sweep" relation was represented reasonably well by a straight line (see Appendix). In fact a straight line through the origin accounted for most of the variation among the points in a graph of the number of flies landing against the number of flies flying. The equation of this line is expressed as:

$$X = bz,$$

where X = number landing, z = number in sweep collections, and b = the slope. The landing activity might be considered a reciprocal of the flying

activity because the flies which land are inactive with respect to flying. The landing activity was studied with varying meteorological factors and was confined to *S. venustum* only, because this species comprised over 90% of the black flies in sweep collections and of those collected as they landed.

The variation in landing activity was investigated first with rate of change of atmospheric pressure. Fewer flies landed when the pressure was rising or falling at 0.01 in. per half hour than when it was almost steady (Fig. 21). This is the inverse of the flying activity as more flies remained actively on the wing when the pressure was changing rapidly. The variations in landing activity with pressure change, although present, were not shown to be statistically significant.

The "landing/sweep" relation was shown by statistics to be represented by a separate straight line for each five degree temperature range from 45-85° F. The variation among the slopes of these lines was too great to be accounted for by experimental error alone. Landing activity was greater at temperatures from 55-65° F. and from 75-80° F. although the latter number was based on many fewer observations (Fig. 22).

The maximum landing activity occurred when saturation deficiency was between 2-4 mm. of mercury and decreased to a minimum at 13 mm. beyond which there was another sharp rise, based however, on fewer data (Fig. 23). This indicates that the greatest landing activity occurred when the air was moderately moist and also again when it was dry. This may be merely a reflection of the effect of moisture content of the air on the flying activity.

The landing activity was greatest at evaporation rates of 0-0.01 units per 10 min. and 0.04-0.05 units per 10 min. on the Piche atmometer (Fig. 24), above which the landing activity on the host decreased. The landing activity at low evaporation rates may reflect the effect of low temperature on the flying activity because low evaporation rates and low temperatures were often coincident. The significance of the second peak is not understood, although it is based on fewer observations than the first. The infrequency of flies landing on the host at high rates of evaporation may be related to the curtailment of flying that would bring the fly to the host in the first place.

The variation in landing activity was studied with simultaneous changes in two meteorological factors. When it was compared with changes in temperature and moisture content of the air, there was one peak of landing activity between 40-60° F. and 2-4 mm. saturation deficiency or 40-70% relative humidity and a second between 75-80° F. and 14-16 mm. (Fig. 25).

The influence of simultaneous changes in temperature and rate of evaporation on landing activity was also investigated. Least landing activity was found at temperatures from 60-75° F. and at rates of evaporation from 0.01-0.04 units per 10 min. on the Piche atmometer. Greatest landing activity occurred at temperatures from 45-55° F. at rates of evaporation of 0.02-0.03 units but at temperatures from 55-65° F. landing activity was greatest when the rate of evaporation was 0.04-0.06 units. Much landing activity occurred also at temperatures of 75-85° F.

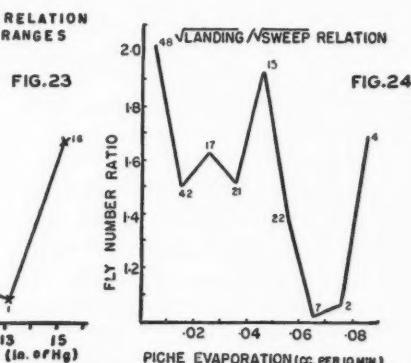
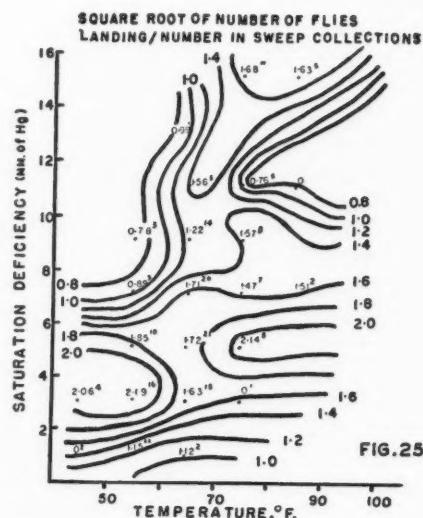
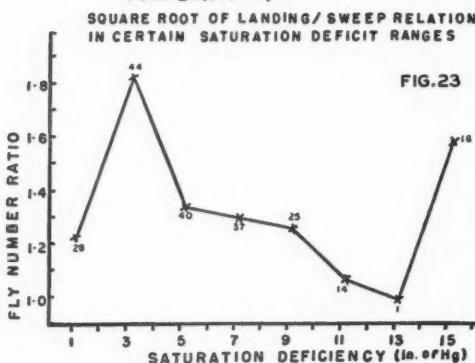
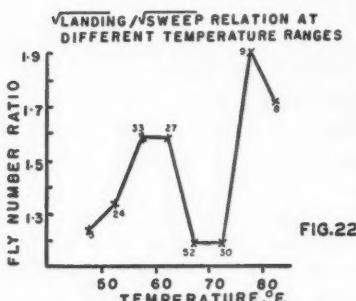
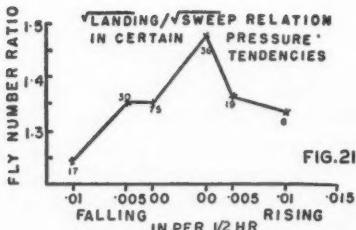


FIG. 21. Square root of landing activity of females of *S. venustum* on human host varying with the change in atmospheric pressure in the half hour preceding the counts.

FIG. 22. Square root of landing activity of females of *S. venustum* on human host varying with temperature.

FIG. 23. Square root of landing activity of females of *S. venustum* on human host varying with saturation deficiency. Correction: Saturation deficiency is measured in "mm. of Hg" not "in. of Hg".

FIG. 24. Square root of landing activity of females of *S. venustum* on human host varying with evaporation rate as measured by Piche atmometer.

FIG. 25. A plan of a three-dimensional graph which represents by isopleths the landing activity of females of *S. venustum* on human host varying with temperature and saturation deficiency.

Variation in Biting Activity with Changes in Meteorological Factors

A straight line relationship was shown to exist between the number of flies biting and landing (see Appendix). The square roots of the numbers were used to make the variance more nearly constant. The biting activity is expressed by the "biting/landing" ratio. This ratio is a more exact expression of activity than the "landing/sweep" ratio because biting and landing counts were made simultaneously and in a similar manner. Again only counts of females of *S. venustum* were used, 99% of the flies collected biting being this species.

It was found that the proportion of flies biting to those landing varied from time to time. This variation in biting activity was studied with six rates of pressure change from 0.01 in. per half hour to near zero. The points representing the number of flies biting and landing were fitted reasonably well by straight lines representing six rates of pressure change. The variation among the slopes of these lines was too great to be explained by experimental error alone (for statistical detail see Appendix). It was shown also that, if these straight lines passed through the origin, that is the point of zero bitings and zero landings, each still accounted for most of the variation among the points. The equations of these lines were of the form:

$$Y = bx,$$

where Y = number biting, x = number landing, and b = slope. Thus there is a series of straight lines radiating from the origin, the slope of each being related to a certain rate of pressure change (Fig. 26). The slopes of these lines exhibit a trend with the greatest slope, the greatest number of flies biting per number landing occurring with large pressure changes per half hour, especially with falling pressures (Fig. 27). For example, with a falling pressure of 0.01 in. per half hour, of 900 flies which landed, 225 bit and, on the other hand, when the change in pressure was near zero, of 900 flies which landed, only 121 bit.

Because these straight lines account for most of the variation among the points, other meteorological factors should exert only a slight influence on the "biting/landing" relation. Actually the changing of other meteorological factors could not be shown to influence the biting activity when the test area was in the shade and receiving only skylight. Some points, showing zero biting for a certain number of flies landing, fell well out of the pattern but no explanation was found by studying the meteorological data for the corresponding times.

The data used in studying the landing and biting activities were obtained from counts of the number of flies landing and biting when the test area of skin was in the shade of the observer's body. However, a few additional experiments were performed to compare the number landing and biting in the intense sunlight and in the shade. The two-minute tests were conducted with the test area alternately in the sun and shade, the observer turning 180° after each test. Intense sunlight reduced the number landing to one-half that in the shade and of those that landed only one-half bit (Table III).

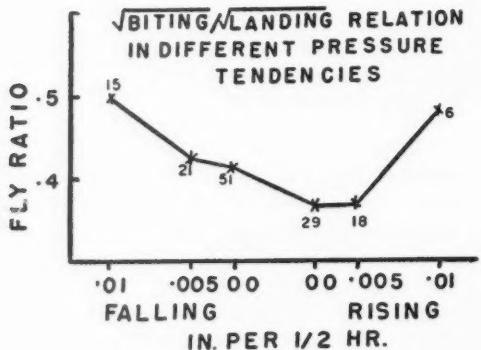
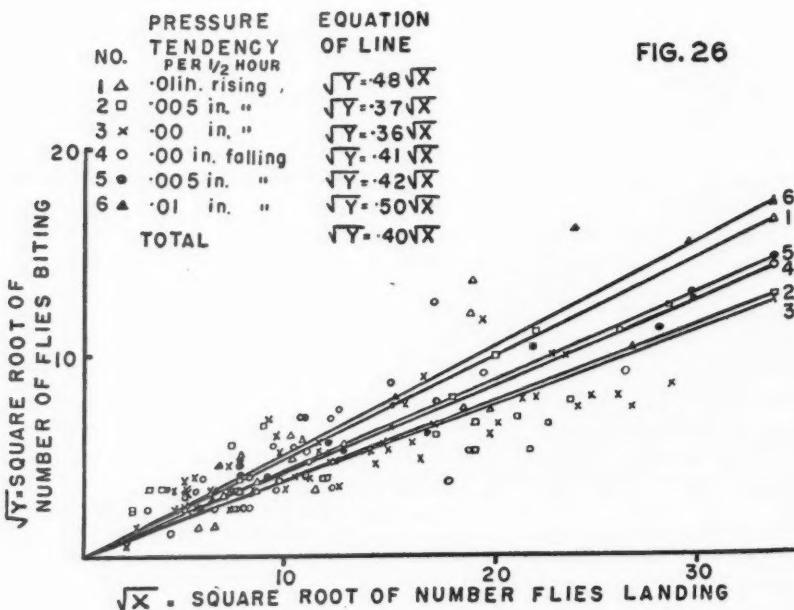


FIG. 26. Square root of the number of females of *S. venustum* biting against the square root of the number landing. Six regression lines are fitted to the points based on the change in atmospheric pressure in the half hour preceding the counts.

FIG. 27. Square root of biting activity of females of *S. venustum* (the slopes of the lines in Fig. 26) varying with the change in atmospheric pressure in the half hour preceding the counts. The number of observations on which each point is based appears beside it.

TABLE III

THE NUMBER OF BLACK FLIES LANDING AND BITING ON AN AREA OF SKIN THREE INCHES SQUARE COMPARED DURING TWO-MINUTE INTERVALS ALTERNATELY IN THE SUN AND SHADE

Activity	Average of 12 tests in sun	Average of 17 tests in shade	Ratio sun/shade
Landing	20.3	42.4	1/2
Biting	1.6	6.3	1/4

DISCUSSION OF THE VARIATION OF ACTIVITY WITH ENVIRONMENTAL FACTORS

Atmospheric pressure change was accompanied by a pronounced variation in the activity of adult female black flies. The greater the pressure change per half hour or per hour, especially with falling pressures, the greater the biting and flying activity. The landing activity on the other hand varied inversely with pressure change. The number of flies on the wing and the number which landed on the host had a bearing on the number which bit. When the pressure is changing rapidly, especially falling, there will be more flies on the wing than when it is steady, thus constituting a large population from which are derived the number of flies landing on the host. Even though a lesser proportion of flies will land on the host at times of rapid pressure change, the total number landing may be greater because of the large number on the wing. A higher proportion of those landing bite when the pressure is changing rapidly than when it is steady. Therefore, because of the greater flying activity as well as the greater biting activity when pressure is changing rapidly, the total number biting will be much larger in rapidly changing than in steady pressure, other factors being constant.

Both Rubtzov (8) and Underhill (12, 13) reported that feeding activity increased during times of falling pressure changes and Underhill indicated that there were more flies swarming around at these times. Conversely he found few flies about and little feeding, when pressure was rising. Wellington (17, 18) observed that the activity of muscoid Diptera increased 30 sec. after the pressure had been lowered by 2-50 millibars, whether the decrease was within two seconds or two minutes, but that activity became normal when the pressure was raised again. In nature if the pressure shows an over-all decrease in a half hour period, then there must have been more falling changes or greater falling changes than rising changes during this period. In general, it was found also in the present study that the greater the decrease in pressure in a period of an hour or less, the greater the number of black flies netted.

The large number of flies, occasionally on the wing at the end of a half hour period when the absolute pressure reading was the same as at the beginning of the period, may be explained by the possibility that there were marked fluctuations in pressure during this period. The greater number of flies netted

with large rising pressure changes may be related to the observation that as the rate of change of pressure increases the number of minor fluctuations on the main pressure pattern increases. This would agree with the observations of Wellington (17, 18) who found that rapid fluctuations in pressure of 2-10 mb. in two to three seconds caused a "frenzied" response in muscoid flies.

The activity of black flies did not vary with absolute pressure, a finding with which Rubtzov (8) agrees. Underhill (12), however, considered that feeding activity was related to absolute pressure being greater between 27.95 to 28.05 in. at 2000 ft. elevation. This is a higher pressure than that found by Wellington (16) for the greatest activity in the Diptera which he studied, i.e. about 26.32 in. He found an increase in activity within the pressure range 29.9-19.4 in.

Temperature is important in influencing the activity of black flies. More flies were on the wing between 60-80° F. than at other temperatures, with most possibly between 75-80° F. Rubtzov (9) reports black flies active from 50-86° F. with greatest activity between 68-73° F. Underhill (12) found *S. jenningsi* Malloch (= *S. nigroparvum* Twinn) most active in feeding on turkeys at a little higher temperature, 75-85° F. In the present study the subsoil temperature was found to be significant also as there was a sharp reduction in flying activity when the subsoil temperature went below 50° F. Landing varied with atmospheric temperature in an inverse manner as compared to flying. Most landing occurred at 55-65° F. with little landing at 65-75° F. Little flying or landing on the host occurred below 55° F. indicating that few flies were active in any capacity at the station at these low temperatures. Temperature may affect the black fly directly or it may act indirectly through its effect on the rate of evaporation of water from the insect.

Moisture content of the air is one of the factors which affects the evaporation of water from the black fly. Most flying activity occurred in moist air, i.e. at saturation deficiencies of 2-5 mm. of mercury or at 70-90% relative humidity but not when it is close to saturation. If observations at temperatures other than from 65-80° F. are included another peak in flying is found in dry air at 9 mm. saturation deficiency or 40% relative humidity, the significance of which is unknown. None of these peaks in activity with moisture content of the air was large. A decrease in flying activity was noted, except for one collection, in saturation deficiencies above 9 mm. and below 40% relative humidity, especially at temperatures over 80° F. Rubtzov (9) found that greatest activity occurred from 75-90% relative humidity. Underhill (13) found no definite relationship between relative humidity and feeding and observed active feeding from 52-94% relative humidity. In previous experiments Underhill (12) feeding was observed at relative humidities as low as 42% with a peak between 65-75%.

Wind may affect the activity directly or indirectly by its effect on the loss of water from the black fly. Winds in the open over 15 m.p.h. were usually accompanied by a decrease in the number of flies on the wing. Considering wind at two feet from the ground near the sweep station, there was most

flying at 50-100 ft. of wind per minute (1 m.p.h.) Uvarov (15) said that small insects, such as Simuliidae, fly only when the air is calm or almost so. Underhill (13) found that winds over 28 m.p.h. reduced activity and that winds of as much as 20 m.p.h. on some days did not, whereas Rubtzov (8) states that speeds over 9-11 m.p.h. (4-5 meters per sec.) will curtail activity of black flies.

Most black flies were on the wing at low, but not zero rates of evaporation. Zero rates are often associated with low temperature which curtails activity and when collections at low temperatures are omitted greatest activity extends to zero rates of evaporation. Insects must maintain their water balance in order to live (14) and thus high rates of evaporation may be detrimental to the black fly. Most of the water loss in insects is a result of evaporation (19). Black flies can open and close their spiracles (6) and much of the evaporation takes place through them. If their oxygen requirement is low as when they are at rest, the evaporation of water from black flies may be minimized by the closure of their spiracles. Wigglesworth (19) referred to a formula devised by Dalton which related rate of evaporation to saturation deficiency. Buxton (2) applied this formula to the rate of water loss from insects. The rate of evaporation depends not only on saturation deficiency but also on the wind and other factors.

Thornthwaite (11) gave a comprehensive discussion of problems of moisture in ecology. He showed evaporation to be related to the vapor pressure gradient from the body, from which evaporation is taking place, to the surrounding air. As the gradient steepens, owing to an increase in temperature of the evaporating surface, the rate of evaporation will increase. This difference between the temperature of the air and that of the black fly would develop when the black fly was in the sun, as insects are poikilothermic. This may explain why fewer flies land and bite in the sun and why fewer flies were on the wing in the middle of the day when radiation and saturation deficiency were high.

We cannot arrive at a true measure of the evaporation from the insect without at least knowing the temperature of the insect as well as the temperature, saturation deficiency, and movement of the air. Other factors such as the activity of the insect, the condition of the spiracles, the shape of the evaporating surface, and the composition of solution from which water is evaporating have some bearing on the rate of evaporation. Therefore, before an exact relationship between insect activity and rate of evaporation can be determined, the influence of the factors mentioned must be known. The evaporating power of the air, as measured by the atmometer, however, indicates probably the approximate range of evaporation rate tolerated by the insect.

Black flies are stimulated by light to fly and a decrease in flying outdoors is usually observed below 275 ft-c. They are attracted to lighted lamps at night and, when indoors, to windows during the day. They do not tend to land as readily in the intense sunlight however, more often selecting the shade

side of the host to land and feed. This increased tendency of black flies to land on dark surfaces, especially of a blue color, was discussed by Davies (5). It may be that light colors attract flies from a distance but that when they reach the host, they select a dark surface on which to land and feed.

The present field study has necessarily focused attention on the effect of meteorological factors on black flies. However, the presence of the human host would influence the number of black flies in the initial sweep collections. Black flies are attracted more by one person than another, but the present observer was less attractive to black flies than were many people. Mer, Birnbaum, and Aioub (7) observed that mosquitoes were attracted by human breath and by air which flowed past the human body. Brown (3) showed that warmth and carbon dioxide gas attracted *Aedes* mosquitoes. The color of the clothing influences attraction, dark blue being most attractive to black flies (5). The physical condition of the present observer depended on his previous activity, a factor hard to standardize in view of the other aspects of the research being conducted. However, because of the large number of observations taken and because of having the same observer in the same outer clothing throughout, these influences are less likely to impose a bias. To further reduce his influence the observer approached the station rapidly and began sweeping immediately. In addition the attraction of the host for black flies was shown not to be influenced greatly by meteorological conditions.

Summary

1. The population of black flies on the wing in the vicinity of Costello creek was measured by a standard method of hand-netting. These "sweep" collections were averaged to reduce individual or daily variations which might have obscured the seasonal change in population.
2. The population of females of *Simulium venustum* Say on the wing varied annually and seasonally coincident with the number and longevity of immigrant and emergent flies. Longevity in turn was probably related to rainfall and to the temperature and saturation deficiency of the atmosphere.
3. Activity was divided into flying, attraction, landing, and biting. The number of flies engaging in each activity was derived from the number in the preceding activity.
4. The number of flies in the individual sweep collection itself (uncorrected for seasonal variation in population) or the difference between the number in each collection and the daily mean number per collection reflected the flying activity.
5. The number of black flies attracted to the observer was determined by comparing the number in the initial collection, made immediately on arrival at the site, with the number in the collection made five minutes later.
6. The numbers of black flies landing and biting on a unit area of human skin in the shade were counted simultaneously in unit time and collections were taken with each series of counts.

7. Females of *S. venustum* comprised 80% or more of the collections of those flies flying, landing, or biting, so that the study of populations and activities has been confined mainly to the females of this species.
8. The flying showed a daily rhythm accompanying periodic variation in meteorological factors, such as temperature, saturation deficiency, and wind speed, which all influence the evaporation rate. There was usually a small peak of flying in the morning and a larger one in the evening. When meteorological factors showed nonperiodic variation, the rhythm in activity was reduced or lost.
9. Attraction varied with none of the meteorological factors measured.
10. Biting, when the area was in the shade, varied only with the rate of change of atmospheric pressure.
11. An increase in the rate of change of atmospheric pressure, especially if the pressure was falling, was accompanied by more flying and biting, but less landing.
12. Atmospheric temperatures of 60–80° F. were accompanied by a peak in flying activity, and least landing on the host occurred between 65–75° F. Flying and landing both decreased to a minimum below 55° F.
13. Changes in saturation deficiency were accompanied by less variation in flying and landing than were other factors. Flying was greatest at low saturation deficiencies, whereas most landing occurred at zero and intermediate values.
14. Winds over 15 m.p.h. in the open reduced flying greatly and in the more sheltered test site the peak in flying activity occurred at 1 m.p.h., especially with winds from the stream and lake margins.
15. Low to zero rates of evaporation were accompanied by the greatest flying activity, whereas landing on the host was greater at moderately high rates.
16. The activities of black flies were little changed by light except at its extremes. There was a gradual decrease in flying below 300 ft-c., although sometimes flying, landing, and biting continued at as low as 1 ft-c. and occurred also under artificial illumination at night. If the shaded area of human skin were exposed to direct sunlight landing decreased to one-half and biting to one-quarter of that in the shade.

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Appendix

The Statistical Procedure Employed in Analyzing the "Biting/Landing" Ratio

This discussion may be divided into two parts as outlined below.

- (1) The use of the square roots of the numbers to reduce the variance (1).
- (2) The variation of the number of flies that bit (y) with the number that landed (x) (10).
 - (i) Can the relationship be explained by a straight line?
 - (ii) Can this relationship be explained by a straight line through the origin?
 - (iii) Does the slope of this line change significantly under different sets of conditions?

(1) The raw data (Table IV) show that, as the average numbers of bites and landings increase, the spread among individual observations gets large, i.e. the variance increases. One may speculate that these counts follow more or less the Poisson frequency pattern, in which case the square roots of the counts should have a reasonably constant variance. When the square roots were applied to the present data, the variances proved to be reasonably steady.

(2) One is tempted to study the behavior of the ratio " y/x ", as a measure of the biting activity of these flies, but before we can interpret properly this ratio, it is necessary to establish the fact that the dependence of " y " on " x " is represented by a straight line which passes through the origin. This enquiry falls into two steps; (i) to establish that the relationship is linear and (ii) to establish that the line passes through the origin. Both steps fall naturally into the arithmetical pattern of the analysis of covariance (10).

Acting on the supposition that the rate of change of pressure has important effects on activity, the (x, y) data were classified into six ranges of pressure tendency (Table IV), and straight lines, $Y = a + bx$, were fitted to the points within each of these ranges. These calculations are exhibited in Table V. The sums of squares and products of deviations from the means, needed to fit these lines and to assess the adequacy of fit, are given under the headings (x^2) , (xy) and (y^2) . In this notation the slope " b " is given by $(xy)/(x^2)$, the sum of squares attributable to the regression line by $(xy)^2/(x^2)$, and the sum of the squares of the deviations from the regression line by $(y^2) - (xy)^2/(x^2)$. These sums of squares are entered in the last two columns of Table V. The smallness of the mean squares computed from the sums of squares of the deviations from the regression line reflects the goodness of fit of the lines to the observed points.

Having decided that straight lines fit the points sufficiently well, the next step is to decide whether straight lines *through the origin* may properly be used. This question is investigated by fitting lines of the form $Y = bx$ to the observations. This is done in Table VI, which differs from Table V only through the fact that sums of squares and products about the origin are entered in the column heads (x^2) , (xy) , and (y^2) . The last two columns of Table VI are filled in exactly the same manner as in Table V, using the same

formulae. The difference between corresponding entries in the last column of Tables V and VI shows the amount by which the goodness of fit of the lines, fitted through the origin, falls below that of lines fitted to allow an intercept. Two of the lines show considerable improvement when an intercept is allowed, but on the whole straight lines through the origin appear to be adequate. The slopes of these lines are shown in Table VII.

The question arises then, as to whether these slopes differ appreciably, or whether on the other hand, we might fit all the points with one straight line. This has to do with the question: has rate of change of pressure any effect on the rate of biting as measured by the ratio y/x ?

This question can be answered by computing the average slope from the totals of (x^2) and (xy) and taking the deviations from it of the individual slopes, or, what amounts to the same thing, by computing the sum of squares of deviations about this average line and comparing it with the total "within groups" sum of squares of the deviations. This is the purpose of the computation at the bottom of Table VI, in which it is seen that the differences among the slopes are too great to be reasonably accounted for by experimental error alone.

TABLE IV

THE SQUARE ROOT OF THE NUMBER OF FLIES BITING (y) AND THE NUMBER LANDING (x) IN CERTAIN RATES OF PRESSURE CHANGE (P)

x	y	x	y	x	y	x	y
<i>(1) P = 0.01 R (rising)</i>				<i>(2) P = 0.005 R (rising)</i>			
19.2	13.4	8.36	3.87	17.3	5.91	7.41	3.16
12.8	5.47	20.0	7.07	21.3	6.70	8.94	6.32
10.8	5.70	5.88	1.40	21.9	5.00	7.74	3.87
19.1	11.8	10.7	4.47	7.73	5.37	22.1	10.4
18.7	7.07	8.36	3.16	12.0	3.87	28.8	12.0
7.74	5.00	7.74	3.16	23.9	7.41	2.23	2.23
6.41	1.52	11.4	3.16	22.8	6.32	2.23	2.23
10.2	5.91			3.16	3.16	18.1	7.74
				11.8	3.87	20.2	9.74
Σx	= 177.39			19.2	5.00	3.87	3.16
Σy	= 82.16			19.2	6.32		
Σx^2	= 2447.37						
Σy^2	= 610.29						
Σxy	= 1171.88						
N	= 15						
(See third to fifth columns in Table V)							
(i) $\Sigma(x - \bar{x})^2 = \Sigma x^2 - \frac{(\Sigma x)^2}{N} = 349.56$							
(ii) $\Sigma(x - \bar{x})(y - \bar{y}) = \Sigma xy - \frac{(\Sigma x)(\Sigma y)}{N} = 200.26$							
(iii) $\Sigma(y - \bar{y})^2 = \Sigma y^2 - \frac{(\Sigma y)^2}{N} = 160.27$							
				<i>(3) P = 0.0 R (rising)</i>			
				16.8	8.66	12.6	3.16
				17.3	7.07	11.1	3.87
				20.4	6.32	9.74	5.00

TABLE IV—Concluded

THE SQUARE ROOT OF THE NUMBER OF FLIES BITING (y) AND THE NUMBER LANDING (x) IN CERTAIN RATES OF PRESSURE CHANGE (P)—Concluded

x	y	x	y	x	y	x	y
(3) $P = 00 R$ (rising)—Concluded							
7.07	3.16	11.6	5.47	8.06	2.23	3.16	2.23
5.00	2.23	14.1	5.00	4.27	1.03	12.2	6.70
16.6	4.72	5.00	3.87	12.7	7.07	10.9	5.00
14.5	5.04	9.48	3.87			10.9	3.87
7.07	2.23	5.47	2.23				
8.36	3.16	4.47	2.23				
14.4	5.47	5.00	3.16				
12.2	4.47	19.7	11.4				
11.6	5.00	23.8	9.74				
6.32	3.16	22.3	7.74				
5.00	3.16	23.0	9.48				
10.4	3.87	2.12	0.028				
10.0	3.16	12.4	4.47				
2.44	1.41	15.2	6.16				
27.0	7.07	9.48	5.91				
29.0	8.12	9.21	6.70				
25.0	7.74	16.1	5.47				
26.2	7.74	24.4	7.07				
5.47	3.87	21.7	7.74				
4.47	3.16	20.0	5.91				
7.07	4.47	7.74	2.23				
15.9	7.28	5.00	2.23				
14.8	5.47						
Σx	= 661.08						
Σy	= 257.35						
Σx^2	= 11156.51						
Σy^2	= 1579.75						
Σxy	= 4060.92						
N	= 51						
(4) $P = 00 F$ (falling)							
11.1	4.47	10.7	6.70	10.7	6.70	22.1	10.0
12.2	3.16	19.3	5.00	12.8	5.00	17.0	5.91
9.74	3.87	19.7	8.94	15.3	7.41	3.87	3.16
7.41	3.16	17.9	3.61	9.21	3.87	30.0	12.2
5.91	3.87	7.41	2.23	7.74	4.47	5.47	2.23
10.3	5.12	8.06	3.87	30.0	12.6	7.74	3.87
17.3	12.2	7.74	2.23	5.00	2.23	7.74	4.47
15.1	8.36	8.06	3.87	6.32	3.16	12.0	5.47
9.48	5.47	7.74	2.23	6.32	3.16	29.3	10.9
26.6	8.94	6.32	2.23				
17.4	7.41	5.47	3.16				
9.48	3.16	26.4	10.9				
(5) $P = 0.005 F$ (falling)							
29.8	15.1	15.4	7.74				
24.2	15.8	6.32	1.73				
6.70	4.47	27.0	10.0				
Σx	= 237.29						
Σy	= 106.81						
Σx^2	= 4491.71						
Σy^2	= 825.88						
Σxy	= 1905.68						
N	= 18						
(6) $P = 0.01 F$ (falling)							
29.8	15.1	15.4	7.74				
24.2	15.8	6.32	1.73				
6.70	4.47	27.0	10.0				
Σx	= 109.42						
Σy	= 54.84						
Σx^2	= 2524.67						
Σy^2	= 660.53						
Σxy	= 1262.42						
N	= 6						

TABLE V

THE CALCULATIONS INVOLVED IN FITTING STRAIGHT LINES $Y = a + bx$, TO THE POINTS WITHIN EACH RANGE OF PRESSURE TENDENCY PER $\frac{1}{2}$ HOUR

Within groups	D.f.	$(x^2)^*$	$(xy)^*$	$(y^2)^*$	Regression		Deviation	
					D.f.	S.S. $(xy)^2/(x^2)$	D.f.	S.s. $(y^2) - (xy)^2/(x^2)$
1	14	349.56	200.26	160.27	1	114.73	13	45.54
2	20	1352.07	359.01	141.96	1	95.33	19	46.63
3	50	2587.36	725.06	281.14	1	203.18	49	77.96
4	28	1001.57	371.47	220.03	1	137.77	27	82.26
5	17	1363.57	497.63	192.08	1	181.61	16	10.47
6	5	529.21	262.32	159.29	1	130.03	4	29.26
Total sum of squares of deviations within groups							128	292.12

* See beginning of Table IV for explanation of these calculations.

TABLE VI

THE CALCULATIONS INVOLVED IN FITTING STRAIGHT LINES $Y = bx$, TO THE POINTS WITHIN EACH RANGE OF PRESSURE TENDENCY PER $\frac{1}{2}$ HOUR

Within groups	D.f.	(x^2)	(xy)	(y^2)	Regression		Deviation	
					D.f.	S.S. $(xy)^2/(x^2)$	D.f.	S.s. $(y^2) - (xy)^2/(x^2)$
1	15	2447.37	1171.88	610.29	1	561.13	14	49.16
2	21	5681.04	2078.77	825.16	1	760.65	20	64.51
3	51	11156.51	4060.92	1579.75	1	1478.16	50	101.59
4	29	5065.77	2102.11	956.98	1	872.30	28	84.68
5	18	4491.71	1905.63	825.88	1	808.52	17	17.36
6	6	2524.67	1262.42	660.53	1	631.25	5	29.28
Total	140	31367.07	12581.78	5458.59	1	5046.73	139	411.86
Total sum of squares of deviations within groups							134	346.58
Sum of squares of deviations of slopes (b 's)							5	65.28

TABLE VII

THE SLOPES OF THE STRAIGHT LINES, $Y = bx$, AND PROOF OF SIGNIFICANT DIFFERENCES BETWEEN SLOPES

Pressure tendency (inches, $\frac{1}{2}$ hour)	Slopes	
	Number	Value $(xy)/(x^2)$
0.01 R	b_1	0.47883
0.005 R	b_2	0.36591
0.00 R	b_3	0.36400
0.00 F	b_4	0.41496
0.005 F	b_5	0.42427
0.01 F	b_6	0.50003

Total = $(\Sigma xy)/(\Sigma x^2) = 0.40111$

Mean square deviation of slopes = $65.28/5 = 13.05$

Mean square deviation from lines = $346.58/134 = 2.596$

$F = 13.05/2.596 = 5.03^*$ with degrees of freedom $n = 5$ and $n_2 = 134$ respectively.

* At 5% level of significance.

The Statistical Procedure Employed in Analyzing the "Landing/Sweep" Ratio

This discussion may be separated into two parts as outlined below.

- (1) The use of the square roots of the numbers to reduce the variance. The explanation given in the preceding discussion on the "biting/landing" relation, is applicable here also.
- (2) The variation of the number of flies that landed (x) with the number that were taken in the sweep collection (z).
 - (i) Can the relationship be explained by a straight line?
 - (ii) Can this relationship be explained by a straight line through the origin?
 - (iii) Does the slope of this line change significantly under different sets of conditions?

One might like to study " x/z " as a measure of landing activity of these flies, but before we can interpret properly this ratio, it is necessary to establish the fact that the dependence of " x " on " z " is represented by a straight line which passes through the origin. The steps involved are similar to those used in studying the ratio " y/x ".

Acting on the supposition that the rate of change of pressure has important effects on activity, the (x, z) data were classified into six ranges of pressure tendency (Table VIII), and straight lines $X = a + bz$ were fitted to the points within each of these ranges. If we follow the procedure outlined in the "biting/landing" study, it is shown that straight lines fit the points sufficiently well. By fitting lines of the form $X = bz$ to the observations, it is shown that straight lines through the origin may be properly used. These calculations are shown in Table IX. It is found, however, that the slopes of these lines, exhibited in Table IX, do not differ significantly although they do show a pattern when plotted against rate of change of pressure. Thus rate of change of pressure in this case may not be as important as other environmental factors in influencing this ratio " x/z ".

Acting on the supposition that temperature has important effects on activity, the (x, z) data were reclassified into eight ranges of temperature (Table X). Straight lines of the form $X = a + bz$ were fitted to the points within each of these ranges. Following the same procedure as mentioned before it was shown that straight lines through the origin would also fit these points. The calculations are shown in Table XI. The slopes of these eight lines for different ranges of temperature, as exhibited in Table XI were found to differ significantly from each other.

TABLE VII

THE SQUARE ROOT OF THE NUMBER OF FLIES LANDING (x) AND THE NUMBER IN THE SWEEP COLLECTIONS (z) IN CERTAIN RATES OF PRESSURE CHANGE (P)

x	z	x	z	x	z	x	z
$P = 0.01 R$ (rising)		(3) $P = 00 R$ (rising)—Concluded					
19.2	13.0	20.0	15.9	5.47	7.41	7.07	4.69
12.8	5.00	5.88	6.82	4.47	3.00	8.36	6.63
10.8	9.89	10.7	8.30	5.00	3.00	14.4	11.0
19.1	12.6	8.36	8.60	19.7	13.8	12.2	11.8
18.7	10.3	7.74	8.88	23.8	18.5	11.6	12.6
7.74	2.64	11.4	9.16	22.3	18.6	6.32	3.16
6.41	4.85	0	0.707	23.0	18.7	5.00	6.63
10.2	4.00	3.87	5.47	2.12	1.73	10.4	8.83
8.36	6.55			12.4	2.00	10.0	10.5
$\Sigma x = 181.26$		9.21		7.21		2.44	
$\Sigma z = 132.67$		16.1		13.2		7.00	
$\Sigma x^2 = 2462.34$		24.4		10.6		29.0	
$\Sigma z^2 = 1281.41$		21.7		14.7		25.0	
$\Sigma xz = 1713.89$		20.0		17.8		26.2	
$N = 17$		7.74		4.89		5.47	
		5.00		0		4.47	
		14.8		8.00		7.07	
		0		1.73		1.58	
		0		1.00		7.07	
		3.87		1.73		0	
		0		1.00		0	
		3.16		2.00		2.23	
		3.87		2.64		6.70	
		0		3.16		3.87	
		2.23		0		0	
		0		3.74		2.23	
		0		1.00		3.16	
		0		1.00		0	
		15.2		10.3		2.23	
		9.48		2.00			
$\Sigma x = 708.75$							
$\Sigma z = 445.37$							
$\Sigma x^2 = 11368.59$							
$\Sigma z^2 = 4375.86$							
$\Sigma xz = 6459.38$							
$N = 75$							

TABLE VIII—Concluded

THE SQUARE ROOT OF THE NUMBER OF FLIES LANDING (x) AND THE NUMBER IN THE SWEEP COLLECTIONS (z) IN CERTAIN RATES OF PRESSURE CHANGE (P)—Concluded

x	z	x	z	x	z	x	z
(4) $P = 0.0 F$ (falling)—Concluded				(5) $P = 0.005 F$ (falling)—Concluded			
12.8	8.48	5.00	1.00	5.00	3.08	12.0	10.0
10.7	9.79	3.16	1.41	6.32	3.46	29.3	20.0
19.3	6.24	0	3.31			1.41	1.00
19.7	4.35	0	4.00				
Σx	= 355.58			Σx	= 238.70		
Σz	= 247.47			Σz	= 169.70		
Σx^2	= 5109.26			Σx^2	= 4493.70		
Σz^2	= 2316.58			Σz^2	= 2030.83		
Σxz	= 3132.68			Σxz	= 2740.43		
N	= 36			N	= 19		
(5) $P = 0.005 F$ (falling)				(6) $P = 0.01 F$ (falling)			
10.7	6.32	22.1	9.27	29.8	25.6	6.32	5.09
12.8	7.54	17.0	19.8	24.2	20.7	27.0	12.4
15.3	13.0	3.87	2.82	6.70	9.27	0.66	0
9.21	13.1	30.0	9.43	15.4	13.6	6.85	4.47
7.74	9.27	5.47	7.48				
30.0	13.8	7.74	8.42	Σx	= 116.93		
5.00	2.64	7.74	9.27	Σz	= 91.13		
				Σx^2	= 2572.03		
				Σz^2	= 1554.39		
				Σxz	= 1932.96		
				N	= 8		

TABLE IX

THE CALCULATIONS INVOLVED IN FITTING STRAIGHT LINES, $X = bz$ TO THE POINTS WITHIN EACH RANGE OF PRESSURE TENDENCY PER $\frac{1}{2}$ HOUR

	D.f.	(x^2)	(xz)	(z^2)	Regression		Deviation	
					D.f.	S.s.	D.f.	S.s.
0.01 R	17	2462	1714	1281	1	2293	16	169
0.005 R	30	5756	3729	2744	1	5068	29	688
0.00 R	75	11369	6459	4376	1	9534	74	1835
0.00 F	36	5109	3133	2317	1	4236	35	873
0.005 F	19	4494	2740	2031	1	3697	18	797
0.01 F	8	2572	1933	1554	1	2404	7	168
Total	185	31762	19708	14303	1	27156	184	4606
Total sum of squares of deviations within groups							179	4530
Sum of squares of deviations of slopes (b 's)							5	76
					$b_1 = 1.3380$	$b_4 = 1.3522$		
					$b^5 = 1.3590$	$b_5 = 1.3491$		
					$b_2 = 1.4760$	$b_6 = 1.2439$		
					$F = 76/5 \div 4530/179 = 0.60^*$			

* At 5% level of significance.

TABLE X

THE SQUARE ROOT OF THE NUMBER OF FLIES LANDING (x) AND THE NUMBER IN THE SWEEP COLLECTIONS (z) IN CERTAIN RANGES OF TEMPERATURE (T)

TABLE X—Concluded

THE SQUARE ROOT OF THE NUMBER OF FLIES LANDING (x) AND THE NUMBER IN THE SWEEP COLLECTIONS (z) IN CERTAIN RANGES OF TEMPERATURE (T)—Concluded

x	z	x	z	x	z	x	z
(6) $T = 70-75^{\circ} F.$				(7) $T = 75-80^{\circ} F.$			
10.7	6.32	23.8	18.5	12.6	4.69	30.0	13.8
12.8	7.54	22.3	18.6	11.1	5.56	17.3	8.24
29.8	25.6	23.0	18.7	9.74	6.32	30.0	9.43
24.2	20.7	7.41	1.41	11.6	7.00	29.3	20.0
15.3	13.0	5.91	2.12	14.1	7.61		
9.21	13.1	5.00	2.64				
11.1	13.3	5.00	3.08				
12.2	9.05	6.32	3.46				
9.74	9.16	7.07	2.64				
6.70	9.27	5.00	2.82				
7.74	9.27	6.32	3.16				
19.2	13.0	5.00	6.63				
4.47	3.00	10.4	8.83				
5.00	3.00	0	1.00				
19.7	13.8	0	1.41				
$N = 30$							
$\sum x^2 = 5364.95$							
$\sum xz = 4314.84$							
$\sum z^2 = 3635.89$							
				(8) $T = 80-85^{\circ} F.$			
				14.8	8.00	0	0.707
				15.9	8.83	2.23	1.00
				16.8	9.59	2.23	2.82
				6.32	5.09	6.85	4.47
$N = 8$							
$\sum x^2 = 850.90$							
$\sum xz = 491.22$							
$\sum z^2 = 289.28$							

TABLE XI

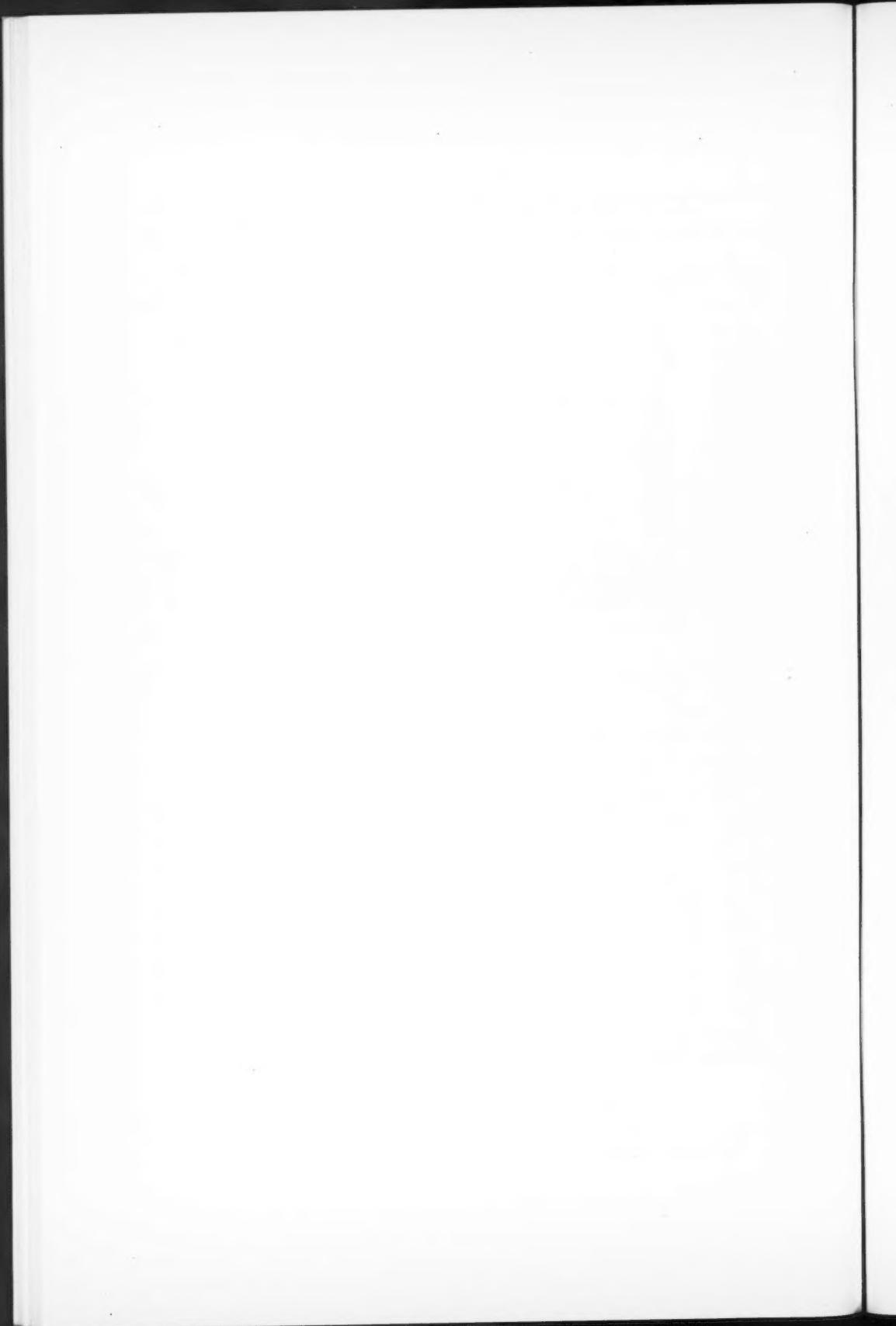
THE CALCULATIONS INVOLVED IN FITTING STRAIGHT LINES, $X = bz$ TO THE POINTS WITHIN EACH RANGE OF TEMPERATURE

Temp.	D.f.	(z^2)	(xz)	(x^2)	Regression		Deviation	
					D.f.	S.s.	D.f.	S.s.
45-50	5	323	402	593	1	500.32	4	92.68
50-55	24	1680	2235	3712	1	2973.35	23	738.65
55-60	33	1732	2747	5260	1	4356.82	32	903.18
60-65	27	2433	3833	6962	1	6038.59	26	923.41
65-70	52	3440	4074	5546	1	4824.85	51	721.15
70-75	30	3636	4315	5365	1	5120.80	29	244.20
75-80	9	947	1796	3668	1	3406.14	8	261.86
80-85	8	289	491	851	1	834.19	7	16.81
Total	188	14480	19893	31957	1	27329.52	187	4627.48
Total sum of squares of deviations within groups							180	3901.94
Sum of squares of deviations of slopes (b 's)							7	725.54
$b_1 = 1.2446$					$b_4 = 1.5754$		$b_7 = 1.8965$	
$b_2 = 1.3304$					$b_5 = 1.1843$		$b_8 = 1.6990$	
$b_3 = 1.5860$					$b_6 = 1.1867$			
$F = 725.54/7 + 3901.94/180 = 4.76^*$								

* At 5% level of significance.

$$\begin{aligned}
 b_1 &= 1.2446 & b_4 &= 1.5754 & b_7 &= 1.8965 \\
 b_2 &= 1.3304 & b_5 &= 1.1843 & b_8 &= 1.6990 \\
 b_3 &= 1.5860 & b_6 &= 1.1867 &
 \end{aligned}$$

$$F = 725.54/7 + 3901.94/180 = 4.76^*$$



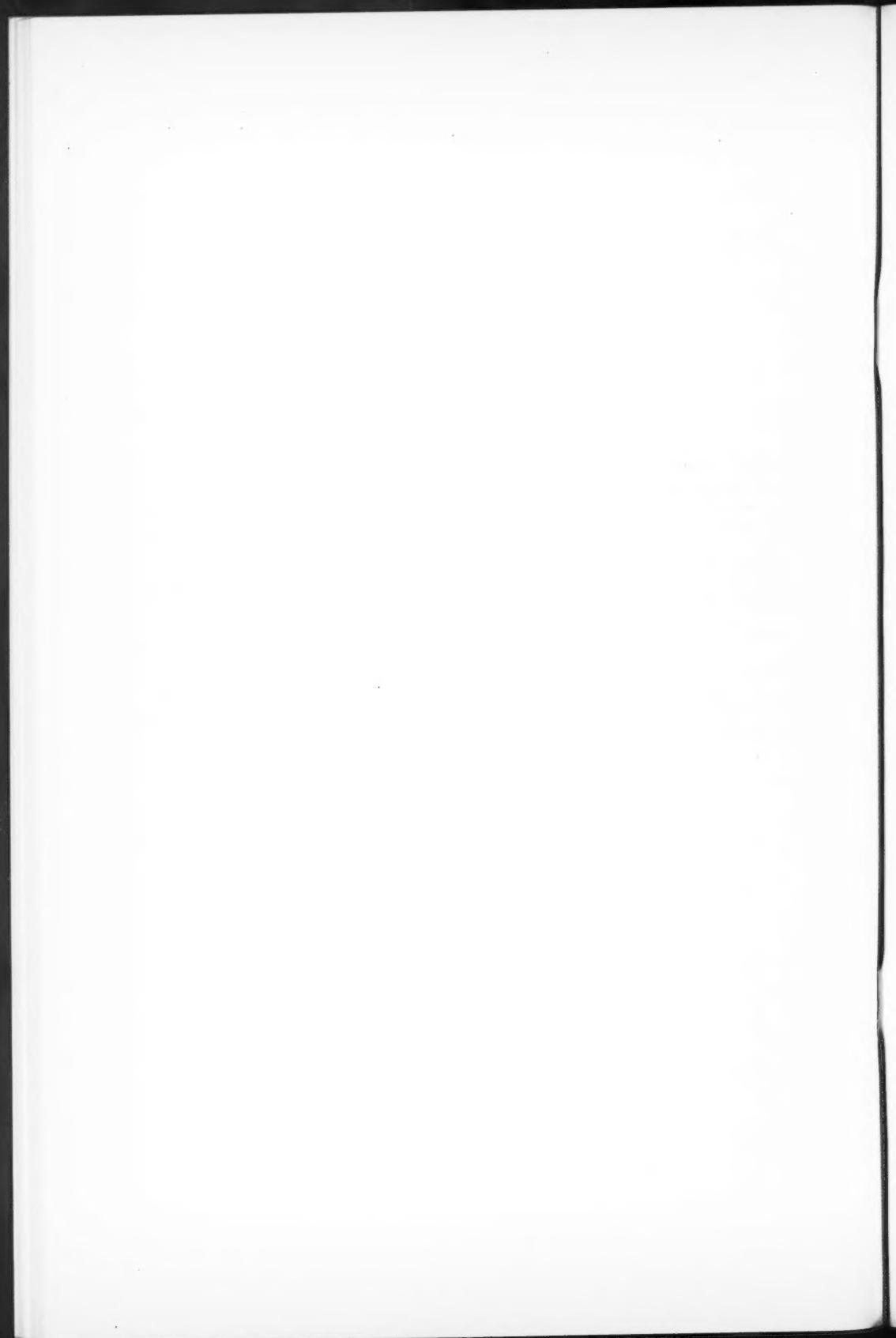
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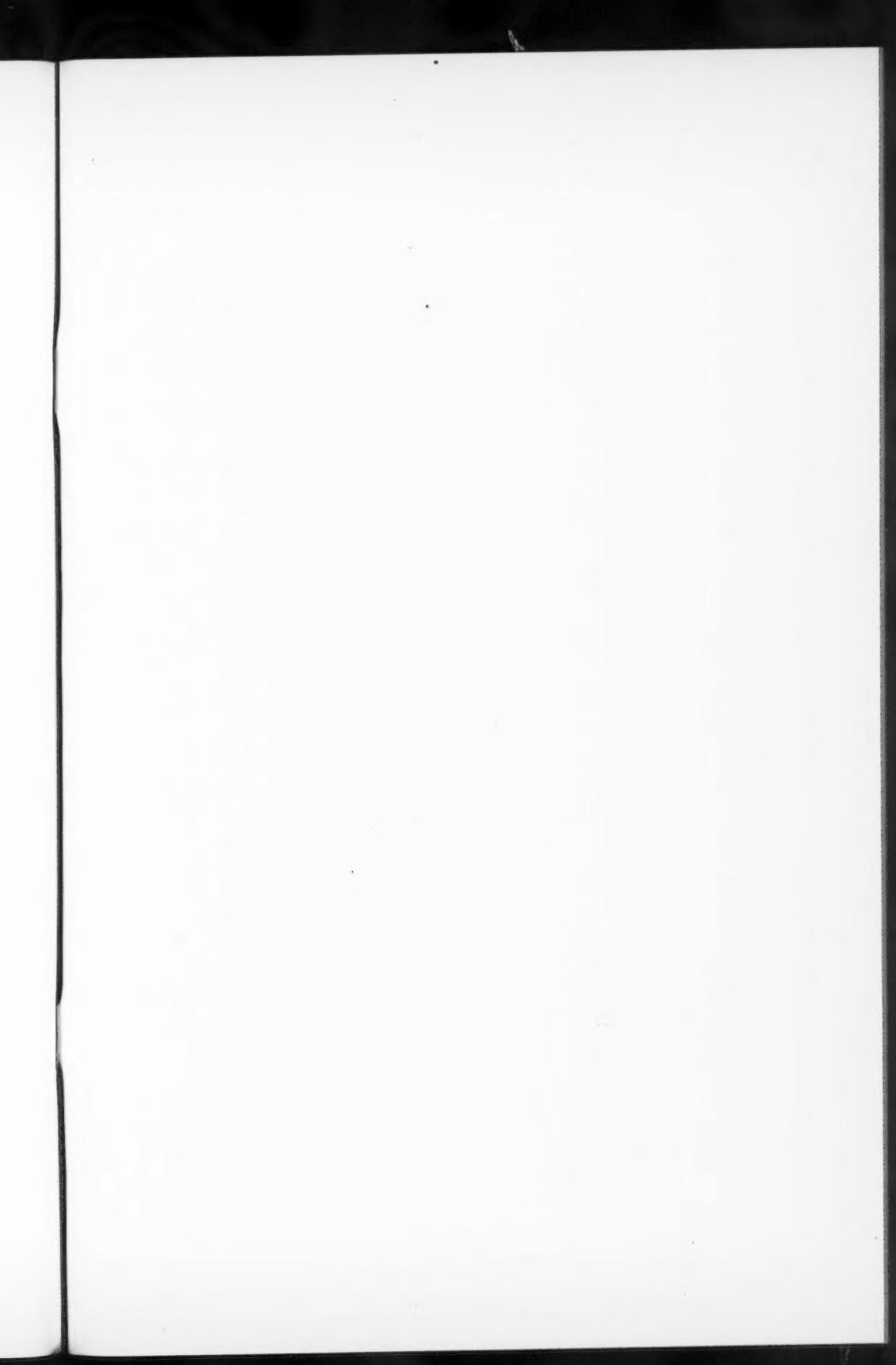
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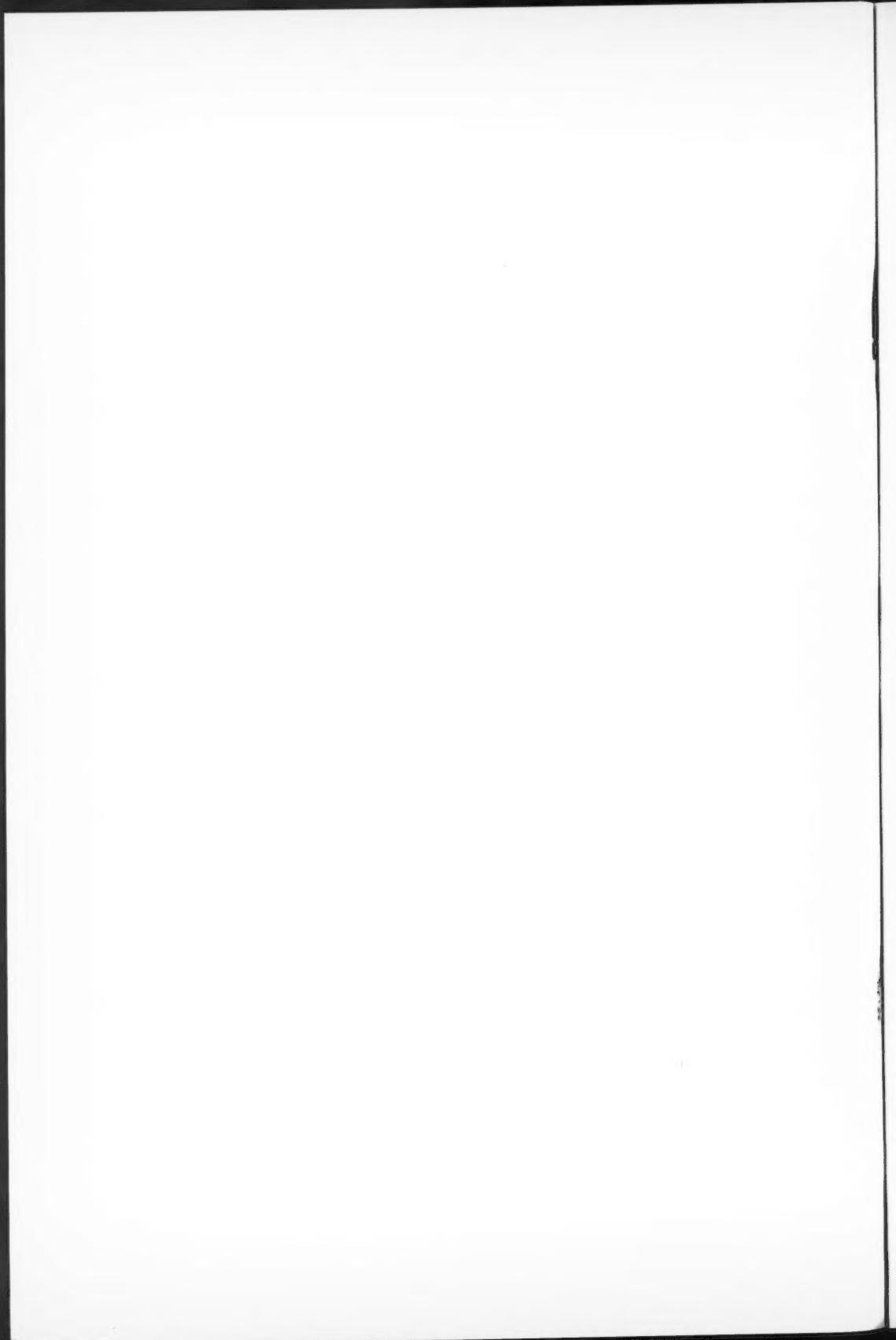
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